

Stefan Arvidsson

ACUTE GASTRIC MUCOSAL LESIONS IN SEPSIS

An experimental study on pathogenic mechanisms



Malmö 1985

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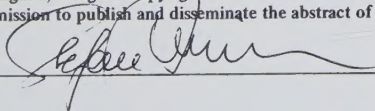
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Title and subtitle Acute gastric mucosal lesions in sepsis. An experimental study on pathogenic mechanisms.			
Abstract Cats were infused with live E.coli (10^9 /ml) for 2-3 h. E.coli alone, or combined with intragastric (i.g.) bile (0.6 ml/kg) or bile and pentagastrin stimulation, was not ulcerative. Sepsis, bile, and exogenous 80 mM HCl always resulted in ulceration of the fundic mucosa. This experimental model was used for further studies in which ulceration was related to gastric (electromagnetic), gastric mucosal (microspheres) blood flow and mucosal regeneration capacity (RNA/DNA ratio). A PGE ₁ analogue (misoprostol, M) was infused i.v. (5 µg/kg/h) or given i.g. (10 µg/kg) 10 min before sepsis. Free radical scavengers, CuZn superoxide dismutase (SOD) and catalase, were infused i.v. (5 mg/kg as a bolus and 50 mg/3 h). A microscopical grading 0-4 of lesions was made in 3 fundic areas giving a lesion index (LI) 0-12 and the number of areas with significant mucosal lesion (grade 2-4). LI in septic cats (n=8) was 8 (7-8) (median) and in sham shock (n=11) 3 (3-4). E.coli induced hypotension, systemic acidosis, a decrease in leucocytes and platelets but no change in gastric or gastric mucosal blood flow or RNA/DNA ratio. LI was >5 in 8/8 E.coli controls, 2/6 given i.v. M, 3/6 i.g. M, and 4/8 SOD/catalase. 92 %, 33 %, 39 %, and 46 %, respectively of the areas had lesion grade 2-4. I.v. M increased gastric blood flow by 120 % and fundic mucosal flow $8+1-25+10$ ml/min/100 g early in sepsis. No further differences were found in gastric blood flow, hemodynamic reactions or blood tests between treated series and controls. It was concluded that septic stress, topical, but not pentagastrin-stimulated, acid and bile were important pathogenetic factors for gastric mucosal ulcerogenesis. Lesions did not develop because of mucosal ischemia or reduced regeneration capacity. Increased blood flow was not the only mechanism by which M was protective. Oxygen free radicals were involved in gastric mucosal ulceration in sepsis.			
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ACUTE GASTRIC MUCOSAL LESIONS IN SEPSIS
An experimental study on pathogenic mechanisms

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Leg. läk. M1m

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ACUTE GASTRIC MUCOSAL LESIONS IN SEPSIS

An experimental study on pathogenic mechanisms

From the Department of Surgery, University of Lund,

Malmö General Hospital, Malmö, Sweden



Malmö 1985

To
Gunilla,
Helen, Therese and Henrik

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I Effects of dihydroergotamine on the feline cardiovascular response to intravenous infusion of live *Escherichia coli* bacteria. S Arvidsson, B Lindblad, C Esquivel, K Fält, C Lindström, D Bergqvist, U Haglund. *Eur Surg Res* 16: 220-231, 1984.
- II Acute gastric ulceration in septic shock: an experimental model. S Arvidsson, U Haglund. *Scand J Gastroenterol* 19 (Suppl 105): 46-48, 1984.
- III Acute gastric mucosal ulceration in septic shock. An experimental study on pathogenic mechanisms. S. Arvidsson, K Fält, U Haglund. *Acta Chir Scand* 150:541-547, 1984.
- IV Gastric Mucosal Damage in Sepsis - Effects of Pretreatment with a Synthetic Prostaglandin E_1 analogue. S Arvidsson, K Fält, U Haglund. *GUT* (in press).
- V Role of oxygen free radicals in the development of gastrointestinal mucosal damage in *E.coli* sepsis. S Arvidsson, K Fält, S Marklund, U Haglund. Submitted.

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INTRODUCTION

Clinical background

Profuse upper gastrointestinal bleeding sometimes appears as a serious complication in the severely ill patient. In the early 19th century Dupuytren (34) and Curling (26) described acute gastroduodenal ulcerations in patients with burn injuries. The association between postoperative sepsis and gastroduodenal bleeding was reported by Billroth in 1867 (10). Later Cushing (27) described the relation between brain injury and acute peptic ulceration.

Initial management of severe traumatized and shocked patients has improved early survival. However, the risk in these patients to subsequently develop multiple organ failure, including acute gastroduodenal ulceration, is high. In the 1960s and 1970s gastroduodenal bleeding in the critically ill patient became a conspicuous problem and much interest was devoted to the pathophysiological mechanisms and to treatment (32, 41, 83, 98, 122).

There are four clinical settings where acute gastroduodenal ulceration often is encountered (see 89, 98, 121).

The first group (stress ulcer) is the most common and includes patients with major trauma, major surgery with complications or prolonged medical illness. These patients develop lesions mainly located in the fundic part of the stomach. The lesions are multiple and superficial, not penetrating the submucosa and without inflammatory reaction.

The second group is patients with burn injuries (Curling ulcer). These patients can have lesions endoscopically quite similar to those of the former group. However, these lesions are also located in the duodenum, and they may be deeper, not seldom complicated by perforation.

The third group is the Cushing ulcer seen in patients with head injuries. These ulcerations are deep and penetrating with a wide distribution from the oesophagus to the duodenum.

The fourth group consists of drug induced or alcohol induced mucosal damage. Even if these lesions often can be difficult to distinguish from other superficial damage, they can hardly be

said to have the same ethiological mechanisms from data available today.

Risk factors for the development of stress ulcer include hypovolemic shock, sepsis and multiple organ failure (89, 122). Of these much attention has been paid to infectious complications and septicemia (mostly gram negative bacteria), which are frequently accompanied by acute upper gastrointestinal ulceration. In such situations the mortality rate may exceed 50 % (3, 41, 48, 122). Bleeding has been reported in frequencies of 12-33 % among operated or traumatized patients with septic complications (4, 43). However, the true figure for the development of mucosal lesions is probably much higher. Serial endoscopic examinations have revealed mucosal lesion within the first few days from the beginning of septic state in up to 100 % of patients (78, 83).

Current concepts of pathophysiological mechanisms

Most of our knowledge about gastric pathophysiology emanates from animal experiments. This has to be considered when discussing the ethiology of acute gastric mucosal ulceration in man. The integrity of the gastric mucosa is dependent on the balance between aggressive and protective factors. Code coined the expression "gastric mucosal barrier" (24), defined as the relative impermeability of the gastric mucosa to Na^+ and H^+ (24, 30). The barrier concept was popularized by Davenport (30) by which the extensive research activities in this field during the last few decades were started.

The integrity of the gastric mucosa can be measured by at least four different means:

1. Morphology. The presence of ulcers and bleeding can be judged macroscopically. For more sophisticated examinations microscopic and electronmicroscopic techniques are requested.
2. "Ion fluxes", i. e. the movement of H^+ , HCO_3^- , Na^+ , K^+ and Cl^- over the mucosa. This is influenced by active secretion-absorption and passive diffusion of anions and cations in either direction.
3. Potential differences across the mucosa, which mostly accompanies changes in "ion fluxes". This way of studying mucosal

integrity has expanded to interest in transmucosal electrical resistance and currents.

4. Biochemical alterations. Changes in RNA/DNA, energy rich compounds, O_2 -metabolism, lysosomal enzymes, cyclic AMP, adenyl cyclase, lipid content of membranes etc. have been identified as indications of mucosal derangement.

However, in discussing the pathogenesis of stress ulcers it must be required that chemical or electrical alterations are associated with morphological damage.

Much research has been devoted to the noxious effects on the gastric mucosa by non-steroid anti-inflammatory drugs and alcohol. It is not evident that it is justified to assume results from such experiments to be relevant in the pathophysiology of acute gastric ulcers in the traumatized or septic patient. Below will be given a summary of different functions and mechanisms, considered to be of importance for gastric mucosal integrity and damage.

Acid. "No acid no ulcer" (119). It has thus for a long time been considered that acid together with pepsin is necessary for acute gastric ulceration to develop (74, 97, 104, 107, 121, 139) and a minimal requirement of gastric luminal hydrogen concentration of 25-50 mmol/l has been demonstrated (71, 96). The gastric mucosa secretes HCl at a maximal concentration of about 160 mmol/l (57) and under normal circumstances the gastric epithelium is capable to resist this acidity. There is a continuous diffusion of H^+ back from the gastric lumen through the mucosa. The rate is different in different species (72) and is linearly correlated to the intraluminal concentration (22, 68). By increased rates of H^+ back diffusion the intramural pH may decrease. This has been associated with mucosal ulceration (68, 71, 127).

Duodenogastric reflux. Guilbert et al (49) found that pylorus-ligated dogs subjected to hemorrhagic shock did not develop gastric lesions in contrast to dogs with an intact pyloric ring. Lysolecithin has been discussed as of importance (69) but most attention has been paid to a direct toxic effect of bile salts

(53). Data on the harmful effect on the gastric mucosa was demonstrated by Davenport (30) who showed that topical bile in dogs increased mucosal permeability for H^+ and Na^+ . Toxic and ulcerative effects have been found experimentally by intragastric bile acid concentrations similar to those obtained in postoperative patients (109). The exact ulcerogenic mechanism is still under debate. The damaging mechanisms of bile include effects on lipid membranes (33), lysosomes (134), mucosal alkali secretion (105), mucosal energy metabolism (95) and gastric mucus consistency (91).

Gastric blood flow. Gastric mucosal ischemia has been implicated, and is generally agreed as a major inciting event in the pathogenesis of acute mucosal ulceration (19, 54, 73, 86, 107, 123). However, hemorrhagic shock alone is, as a rule, not capable of inducing mucosal ulceration or even disturbances in transmucosal potential difference and ion fluxes (31, 106). Gastric ischemia may act aggressively on the mucosa and result in intracellular hypoxia of the superficial epithelium (13) and disturbed mucosal energy metabolism (90, 94). It may, however, instead of being an aggressive factor also act by reducing the mucosal defence capacity. Blood flow removes or neutralises hydrogen ions from the mucosal tissue (127). Experimentally increased blood flow is demonstrated to protect from acute gastric ulceration (99, 110). A critical ratio of back diffusing H^+ to mucosal blood flow has been proposed above which there is a high risk of ulcer development (99, 108).

The "mucus bicarbonate" barrier. The superficial epithelium of the antral and fundic mucosa secretes bicarbonate (see 40). The bicarbonate is supposed to diffuse towards the lumen through the overlying mucus glycoprotein layer and neutralize back diffusing H^+ . A significant pH gradient has been demonstrated in the mucus layer (7, 137). The combination of mucus and buffer - the "mucus-bicarbonate" barrier - constitutes an important tool for the preservation of gastric mucosal integrity (2, 133, 137). However, the magnitude of alkali secretion is estimated only to 5-10 % of the maximal acid output (39). The question arises whether this relatively small amount of buffer, provided by the superficial cells, is the only neutralizing factor. It has been

found that the pH gradient can persist with intraluminal pH of about 2-3 (70, 114, 133).

Regeneration and reconstitution. The superficial layer of the gastric mucosa originates from the glandular neck cells with a turnover rate of 2-4 days (81, 129). Early reduction in mucosal DNA synthesis and RNA concentration is found in response to stress and is accompanied by gastric mucosal ulceration. (59, 84, 132).

Superficial lesions of the gastric mucosa is known to heal rapidly, within 24 h, in contrast to a somewhat deeper damage (138). Recently, it has been found that if the superficial part of the frog gastric mucosa is desquamated by hyperosmolar NaCl, it may be covered within minutes by epithelial cells. Complete restoration of epithelial continuity was found in 4-6 h (130). It has been concluded that this was not caused by regeneration but, possibly, by a migration of mucous neck cells: reconstitution (130) which was dependent on luminal pH and only seen with pH above 3 (131).

Oxygen free radicals. Most of the oxygen consumed in the body is directly reduced to water in the mitochondrial cytochrome chain. However, a few per cent are reduced through other mechanisms leading to the formation of a series of toxic oxygen reduction products. In sequence the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) are formed (see 42, 92). Oxygen derived free radicals are involved in tissue damage in various organs (16). In the gastrointestinal tract free radicals are associated with mucosal lesions in the small intestine following regional ischemia and reperfusion (102, 118). The importance of free radicals in the gastric mucosal damage has not been explored but reduced ulcer formation in cold and restraint stressed rats by a scavenger of free oxygen radicals has been reported (23).

Mediation of mucosal defence. The mucosal defence can be delineated in four parts. From the luminal side these are the "mucus-bicarbonate" barrier, the alkaline tide, the reconstitution phenomenon and blood flow (nutrients and buffer supply).

The first line has been proposed to be mediated by prostaglandins. Prostaglandins are produced in the gastric mucosa with various effects on gastric functions (see 65). Exogenous supply of synthetic prostaglandin E-analogues inhibit gastric acid (20, 75, 113) and pepsin (29) secretion. Prostaglandins in doses without effects on acid inhibition protect the gastric mucosa from damage caused by noxious agents like boiling water and concentrated ethanol (112). The exact mechanisms behind this effect, called "cytoprotection", are not fully understood (see 65, 111). Prostaglandins possess the ability to increase mucus synthesis (62) and secretion (11) as well as bicarbonate secretion (37, 45, 64, 66). A stabilizing influence on transmucosal potential differences and ion fluxes (25, 28), on lysosomal membranes (38), as well as trophic effects on surface epithelium and mucous neck cells (63) and an increase in gastric mucosal levels of surface-active phospholipids (80), has been demonstrated by exogenous prostaglandin supply.

Prostaglandins protect the gastric mucosa from damage due to concentrated HCl, NaOH or ethanol and boiling water (112). Microscopical examination of the gastric mucosa following topical absolute ethanol and prostaglandins has revealed that, even if gross damage is abolished, the superficial mucosal cell lining is not intact. The depth of the damage is reduced, necrosis and hemorrhagic lesions are minimal with pretreatment with prostaglandins (76). This suggests effects of prostaglandins not only on the superficial cell layer but also in the deeper parts of the mucosa.

AIM OF THE STUDY:

- To develop an experimental model where live E.coli bacteriemia regularly induces gastric mucosal lesions,
- to examine the relative importance of intragastric hydrochloric acid and bile in the development of such lesions,
- to relate the development of acute gastric mucosal damage to small intestinal mucosal lesions in live E.coli bacteriemic shock,
- to study gastric mucosal blood flow in relation to acute mucosal ulcerations,
- to investigate the possible importance of the regeneration capacity of the gastric mucosa as a factor in the pathophysiology of mucosal damage,
- to study if a prostaglandin E_1 -analogue may influence the development of lesions and, if so, by which mechanism, and
- to investigate the possible participation of oxygen derived free radicals in the formation of acute gastric mucosal damage in sepsis.

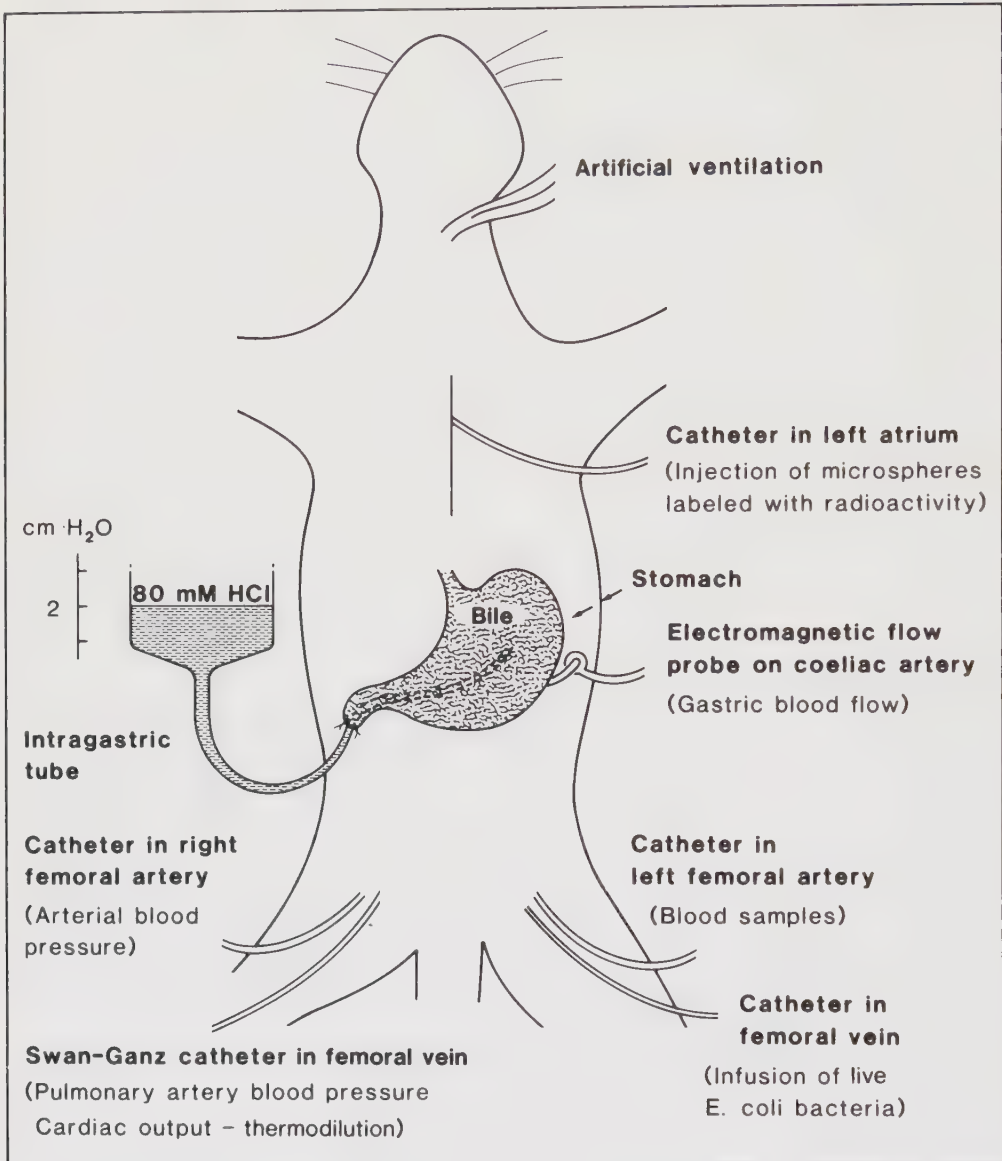


Fig 1. Illustration of the experimental model used in papers III-V.

METHODOLOGICAL CONSIDERATIONS

Material and methods are described in detail in papers I - III. Below will be given a short survey and comments.

Experimental animals

The experiments were performed on 73 healthy cats weighing 1.9-5.0 kg. The animals were deprived of food for 24 h but had free access to water. Cats were chosen as the experimental animal because of similarities with humans in the splanchnic circulation (58, 60). Furthermore, much data are at hand in splanchnic pathophysiology and septic shock (35, 36).

Anesthesia

Anesthesia was induced by 50 mg pentobarbital (PENTHOTHAL^R) i.v. and continued with chloralose (50 mg/kg) i.v. given as a single dose. The animals were artificially ventilated with room air and a tidal volume of 15 ml/kg b.w. at a frequency of 18/min.

Operative procedures

In paper III a detailed description of the operative procedures used in papers III - V is given (for illustration see fig 1). Polyethylene catheters were introduced into the femoral arteries for blood sampling and monitoring of mean arterial blood pressure. Infusions were given in the femoral and brachial veins. A Swan-Ganz catheter, placed with its tip in the pulmonary artery, was introduced through the right femoral vein for monitoring pulmonary artery blood pressure and cardiac output by thermodilution. A left parasternal thoracotomy was performed, the pericardium cut open and a catheter was placed into the left atrium of the heart secured by a purse string suture. This catheter was used for injection of microspheres (see below). Following laparotomy and splenectomy a gastric tube was introduced through a duodenotomy and connected to a reservoir, keeping the intragastric pressure constant at 2 cm of water. The tube was fixed with a ligature around the pylorus, thus preventing duodenogastric reflux during the experiment. An electromagnetic flow probe was placed around the coeliac artery. The hepatic artery was ligated proximal to the gastroduodenal artery allowing collateral circulation to the liver.

The animals in paper I were subjected to a less operative challenge in that no laparotomy was performed. In paper II, with the exception of three cats, no thoracotomy was done. The Swan-Ganz catheter and the electromagnetic flow probe were not used either.

Experimental setting

After a stabilisation period of at least 30 min basal hemodynamic values were obtained. In papers III - V gall-bladder bile (0.6 ml/kg b.w.) and 80 mM HCl were instilled into the stomach through the duodenogastric tube in all cats 1 min before the start of bacterial/saline infusion. Live *E.coli* bacteria in a concentration of 10^9 /ml were infused at a rate of 1 ml/kg/min for 2 min and then 1 ml/kg/h for 2 h in papers I, II, and 3 h in papers III - V. Then the experiments were terminated. Sham controls (I, III) were given an equivalent amount of saline. A glucose solution containing bicarbonate was infused (10 mmol NaHCO_3 /100 ml in 5.5 % glucose; 0.1-0.2 ml/min) to compensate metabolic acidosis secondary to the surgical preparation (51).

Blood flow

Cardiac output was measured by thermodilution. The Swan-Ganz catheter (5F, Edward Laboratories, Santa Ana, California, USA) was connected to an Edward cardiac output computer 1520. Cooled (0-1°C) 5.5 % glucose solution was injected in a volume of 2 ml/registration. With the exception of the basal value, which was calculated from the mean of 2-3 injections, only one injection was made at each registration. This way of monitoring cardiac output correlates well to registration by the microsphere technique (5).

Gastric blood flow was continuously obtained by an electromagnetic flow probe (Model CF406; Cliniflow 601 D, Carolina Medical Electronics Inc, King, North Carolina, USA) on the coeliac artery. Zero level was checked about every 30 min during the experiments. Following splenectomy and ligation of the hepatic artery proximal to the gastroduodenal artery, coeliac artery blood flow would approximately give total gastric blood flow. Basal hepatic blood flow in paper I (without artery ligation) was 18 ± 6 to 25 ± 10 ml/min $\times$ 100 g and in papers III - V (with hepatic artery ligation) 19 ± 3 ml/min $\times$ 100 g (n=34). Thus, ligation of

the hepatic artery proximal to the gastroduodenal artery did not significantly reduce the hepatic arterial inflow.

Tissue blood flow. Three different radioactively labelled microspheres (^{46}Sc , ^{103}Ru , ^{141}Ce ; $15\pm 3\ \mu\text{m}$, NEN) were injected at random order before, at early (15 min) and at late (150 min) sepsis (III, IV, V) or 3 and 120 min after sepsis induction (I). If the arterial blood pressure fell to a level of $<50\ \text{mmHg}$, the last injection was made at that time. The times of injection are therefore referred to as "early" and "late" sepsis. Before injection the microspheres were vigorously shaken in a mechanical shaker. Reference blood flow was withdrawn from the thoracic aorta at a speed of 2.2 or 3.4 ml/min starting 10 sec before injection, continuing for a total time of 70 sec. The reference blood sample was dissolved in three test-tubes containing 3 ml conc. sulphuric acid each.

At the end of the experiments pieces of the gastric wall were excised from the fundus, the major curvature and the antrum. The mucosa (appr. 1-2 g) was peeled off and dissolved in 3 ml conc. sulphuric acid. The radioactivity was measured in an automatic gamma-scintillation counter (Ultragamma^R). For fundic mucosal blood flow the mean activity of the fundic and major curvature samples was used. Antrum flow was calculated from one tissue sample only. All other measurements of regional blood flow were calculated from the mean of two different tissue samples. Tissue blood flow (Q) was given by the formula (55):

$$Q = 100 \times \text{tissue radioactivity (counts/min} \times \text{g)} \times \text{reference blood flow (ml/min)} \times \text{reference radioactivity (counts/min)}^{-1}$$

Q is then expressed as ml/min $\times$ 100 g. Reference blood flow was 2.2 or 3.4 ml/min.

The microsphere technique for measuring tissue blood flow is based on a direct proportionality between the number of microspheres entrapped in the tissue and blood flow to the organ (136). The validity of the technique is dependent on several factors as reviewed by Heymann. Adequate mixing in the aorta was checked by calculating the correlation coefficient for flow in

the right and left kidney (55). The equation based on the recordings in papers III-V was $y=1.05x-5.45$; $r=0.90$. In paper III the number of spheres, on which each calculation of fundic blood flow was based, was 1387 (range 300-5923; $n=45$). The corresponding values for the antrum were 342 (57-1895). The electromagnetic gastric blood flow recordings were used to ensure that the microsphere measurements were made at representative periods of the experiments.

Histological examination

With the exception of paper II microscopical grading of mucosal damage was chosen since damage to the mucosa cannot always be detected macroscopically (76). After removal of the specimens for gammacounting and biochemistry, the remainder of the stomach was mounted on cork and fixed in 4 % formalin. Three standardized areas of the fundic part of the stomach, (the anterior wall, the posterior wall and the major curvature), were predetermined for histological examination. The blocks were prepared for paraffin embedding, cut at 3-4 μm , mounted and stained with hematoxylin-eosin. The glasses were coded for histological examination and analyzed by the pathologist without further information on details about the experiments. The gastric mucosal lesions were graded 0-4 (III) where

0=normal mucosa,

1=subepithelial edema,

2=desquamated superficial epithelium,

3=damage of the upper half of the glands,

4=disappearance of the glands.

By using this grading system a gastric mucosal lesion index was calculated as the sum of the maximal grade in each of the three areas. This index relates to the mucosal depth and spread of the lesions.

It was felt that damage graded 2-4, that is disrupted superficial epithelium, constituted the significant mucosal damage and that the index system could hide a difference in distribution of such damage. The spread of significant mucosal lesions was estimated as the total number of areas with and without intact epithelium.

Small intestinal specimens were taken about 20 cm from the liga-

ment of Treitz, cut open, mounted on cork, fixed in formalin for histological preparation and graded using a scale 0-5 (1, 21) (I, V). Colonic specimens were excised from the mid-portion of the colon. They were prepared in the same way as described for the small intestine. A grading 0-5 was made (V).

Blood tests

White blood cells and platelets were counted in a Bürger chamber and arterial pO_2 , pCO_2 and pH were measured in an acid-base analyzer (ABL 1, RADIOMETER A/S, COPENHAGEN, DENMARK) before sepsis, at early and at late sepsis (I: 5, 60, 120 min; III-V: 15, 70-150 min).

SOD was determined in the terms of its ability to catalyze the disproportionation of $O_2^{\cdot -}$ in alkaline aqueous solution.

The disproportionation was directly studied in a spectrophotometer (87) (V). Plasma levels of catalase were measured by the rate of H_2O_2 disproportionation at 240 nm in a cuvette containing 10 mM H_2O_2 in 3 ml 10 mM K phosphate, pH 7,4 + 0.1 mM diethylenetriaminepenta-acetic acid (V).

Collagen determination

Before sepsis induction a 5x5 mm, total wall piece was taken from the anterior wall of the gastric fundus. After sepsis pieces were obtained from the corresponding areas of the posterior wall and from the major fundic curvature. The mean of these values was used for collagen concentration in the gastric wall after sepsis. The specimens were immediately frozen to $-18^\circ C$ for later analysis. All specimens were dried in an oven at $100^\circ C$ to constant weight. Collagen was determined as hydroxyproline after hydrolysis (6 N HCl for 3 h at $130^\circ C$) as previously described (128). The hydroxyproline concentration was expressed as $\mu g/mg$ dry weight.

RNA/DNA concentration

Determination of RNA (18) and DNA (17) was made in 5x5 mm large pieces of the gastric mucosa obtained at the same time and from the same areas as the collagen biopsies. Standards were delivered from Sigma Chemical Company, St Louis, USA. RNA and DNA concentrations were expressed as $\mu g/100$ mg wet weight.

DNA duplication and mitosis is preceded by an increase in RNA synthesis (8). If DNA concentration is supposed to correlate to the number of living cells in the tissue, then variations in RNA would reflect the capacity of the mucosa to generate DNA before cell duplication. The ratio RNA/DNA was measured with the implication that it reflects the regeneration capacity of the gastric mucosa.

Bacterial preparation

Live *Escherichia coli* bacteria (strain E.coli 06 K13 H1) (WHO designation Su 4344/41) from the WHO Collaborative Center for Reference and Research on *Escherichia* (Serum State Institute, Copenhagen, Denmark) were used. The bacteria were cultivated in ordinary nutrient broth. Just prior to infusion the bacteria were washed in saline, resuspended and infused in 0.9 % NaCl. A concentration of 10^9 bacteria/ml was chosen. Terminal arterial blood cultures, obtained in some experiments, were regularly positive for the growth of this E.coli strain.

Statistical methods

Values are expressed as mean $\pm$ SE if based on 5 or more observations, otherwise the mean value alone is given. In ordinal scale data median and interquartile range (i.r.) are used. Statistical significance is calculated by Wilcoxon matched-pairs signed-ranked test, Wilcoxon rank sum test or Fisher's exact probability test (120). A p-value of <0.05 was considered as significant.

Drugs

1. Dihydroergotamine (DHE): ORSTANORM^R (Sandoz AB, Täby, Sweden). DHE is an unspecific receptor blocker with effects on alpha-adrenergic and serotonergic receptors. Furthermore, DHE has a partial agonistic effect on the alpha-adrenergic receptors (116). The vasoconstriction induced by DHE mainly affects the capacitance vessels and to a lesser extent the precapillary resistance vessels (93).
2. Ketanserin (Janssen Division, AB Leo, Sweden) is a specific serotonin₂ receptor blocker (79, 135).

3. Pentagastrin: PEPTAVLON^R (Imperial Chemical Industries, Macclesfield, England). Pentagastrin is a potent stimulator of gastric acid secretion.
4. Misoprostol (SC-29333 G.D. Searle & Co Ltd) is a 15-deoxy-16 hydroxy-16 methyl analogue of prostaglandin E₁. Misoprostol was stored at -80 in absolute ethanol at a concentration of 10 mg/ml and just before use dissolved in phosphate buffer (pH 7.4) for infusion and in 1 ml saline for topical administration (IV).
5. Superoxide dismutase (SOD): (yeast CuZn SOD; Pharmacia AB, Uppsala, Sweden). Superoxide dismutase disproportionates the superoxide anion radical to hydrogen peroxide and oxygen ($2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2$). The drug was dissolved in 3 ml 0.1 M TRIS HCl buffer with pH 9.5 (V).
6. Bovine catalase (Boehringer Ingelheim AB, Skärholmen, Sweden) disproportionates hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow O_2 + 2H_2O$). Catalase was dissolved in the same way as SOD.

Experimental design

To facilitate the surveyability of the different experimental series used in this study the following presentation is given:

Study I

E.coli were infused during 2 h in three groups of cats:

- 1) Bacteria alone (n=4)
- 2) DHE (0.02 mg/kg x b.w.) was injected as a bolus 5 min prior to bacteria (n=6).
- 3) Ketanserin (0.25 mg/kg x b.w.) was injected 5 min before bacteria (n=6).

In addition two cats received the same volume of saline (sham shock).

Study II

Live *E.coli* were infused for up to 2 h in four groups of cats:

- 1) Bacteria only (n=2)
- 2) Gallbladder bile (0.6 ml/kg x b.w.) was instilled into the stomach 1 min before sepsis induction (n=4).
- 3) Pentagastrin (Peptavlon^R, 16 µg/kg) was infused during 45 min. The infusion was terminated 15-30 min prior to bacterial infusion. Bile was given as described above (n=6).
- 4) Bile, as well as 80 mM HCl, was administered in the stomach 1 min before sepsis (n=7).

Study III-V

Four groups of cats were infused with *E.coli* for up to 3 h. Sham shock animals received saline. Intragastric bile and HCl was given in all cats.

- 1) Bacteria alone (controls; n=8).
- 2) Misoprostol (5 µg/kg x h) was infused i.v. continuously starting 10 min before bacteria (n=6).
- 3) Misoprostol (10 µg/kg) was administered intragastrically 10 min before bacteria (n=6).
- 4) SOD was given as an i.v. bolus (5 mg/kg) 2 min before bacteria followed by a continuous i.v. infusion (50 mg/3 h; n=8). Of these cats four also received catalase in the same dose as for SOD.
- 5) Sham shock (n=11).

RESULTS AND COMMENTS

General reactions

Arterial blood pressure decreased in sham shock cats during the last hour of the experiments. This can be ascribed to the operative trauma. Systemic arterial blood pressure decreased in all groups given bacteria, except in DHE pretreated animals. In that group blood pressure increased before bacteria remaining at a higher level during bacterial infusion (I). Ketanserin decreased blood pressure before bacteria. It was then maintained fairly constant during 2 h of septic state (I). Misoprostol or SOD/catalase had no particular effect on blood pressure.

Pulmonary arterial blood pressure increased by more than 100 % with maximal values 3 min after bacteria. It was nearly always normalized at 15 min (I, III). Pulmonary vascular resistance increased temporarily 4-6 times early in sepsis. It never normalized completely after the initial period but returned to a level of about 70 % above the preseptic value (I).

Cardiac output responded biphasically with an initial increase during the first few minutes after the start of bacterial infusion, followed by a slow decrease throughout the experiments. In late sepsis cardiac output was about 60 % of the preseptic value. Except that the early response was not found in cats pretreated with scavengers, the drugs given did not change the septic influence on cardiac output (III, IV, V). In paper I, where the animals were subjected to a less extensive operative trauma and a shorter period of sepsis, cardiac output did not change significantly, except for a slight decrease in DHE-treated animals.

The number of circulating leucocytes in arterial blood decreased at early and more pronounced at late sepsis. It was mainly due to a decrease in polymorphonuclear white cells to 15 ± 5 % of the pre-septic value ($n=12$). The concentration of platelets in arterial blood decreased to 43 ± 6 % of basal early in sepsis but also in cats subjected to sham shock (61 ± 5 %; I, III). The number of circulating platelets tended to normalize at late sepsis. Systemic pH declined significantly from 7.44 ± 0.03 to 7.32 ± 0.05 ($p < 0.05$) in septic cats. pO_2 did not change during septicemia. Prebacteri-

emic $p\text{CO}_2$ was $3,82 \pm 0,13$ indicating a slight hyperventilation. The responses in blood cells and systemic pH was not influenced by ketanserin, DHE, misoprostol or SOD/catalase. It was concluded that the drugs given did not influence the general hemodynamic reactions or the results of the performed blood tests during live *E.coli* infusion.

Gastric mucosal damage

Influence of bile - acid - sepsis. In the present series of experiments sepsis alone did not induce gastric mucosal damage (I, II). *E.coli* bacteriemia and topical bile or topical bile and pentagastrin-stimulated acid secretion did not induce gastric mucosal damage. In septic cats 80 mM HCl - giving an intragastric pH of about 1 as did pentagastrin stimulation - induced significant macroscopical mucosal damage if instilled into the gastric lumen together with bile. In contrast, live *E.coli* bacteriemia alone induced significant lesions to the small intestine: grade 2-5 in 13 of 16 (I) and 4 of 8 (V) animals respectively. The gastric ulcerations were not due to low blood pressure only, since a profound hypotension with end arterial pressures of 63 ± 21 and 40 ± 12 mmHg was found in cats with intact mucosa and 43 ± 17 mmHg in cats with macroscopical ulceration (I, II).

All macroscopical gastric lesions were located in the gastric body while the antral area appeared normal. The mucosal damage (long fissures or confluent areas with or without bleeding) was as a rule found at the major curvature but not at the minor. The areas selected for histological study (the major curvature, anterior and posterior fundic wall) thus represented the sites most often damaged.

On the microscopical examination it was found that the damage started at the apical part of the mucosa with a subepithelial edema, followed by a desquamation of the superficial epithelial cells (III). Progressive necrosis occurred down the glands with the ultimate disappearance of glandular tissue. There was no accumulation of inflammatory cells. This series of events is similar to that reported in dogs subjected to septic shock (104).

Gastric blood flow. Approximate total gastric blood flow, measured electromagnetically, was 32 ± 3 ml/min ($n=37$) before bacteria /saline infusion and before drugs (III, IV, V) and it was virtually constant during 3 h of septicemia. In cats not given bacteria (sham shock), flow increased by 15 % during the first 5 min followed by a decrease late in the experimental period to levels below that of septic cats at 120 min (18 ± 2 vs 26 ± 6 ml/min respectively).

Basal values for gastric mucosal blood flow, measured by microspheres, ranged from 5.7 ± 0.9 to 17.1 ± 4.8 ml/min $\times 100$ g in the fundic area (I, III, IV, V) and for antrum from 5.4 ± 0.8 to 13.4 ± 4.8 ml/min $\times 100$ g (III, IV, V). There were no significant alterations in mucosal blood flow during the bacteriemic or sham shock period, nor were there significant differences between sepsis and sham shock, nor between antral and fundic mucosal blood flow (III). There was only a weak correlation between gastric lesion index and fundic flow at late sepsis ($r=0.58$). In the small intestinal mucosa, even if mucosal blood flow decreased in some groups, no correlation was found between lesion grade and flow.

Metabolic events and mucosal regeneration capacity. A correlation was found between low arterial pH and gastric mucosal damage at late sepsis ($r=0.69$, $p<0.01$). Gastric mucosal regeneration capacity, measured as the RNA/DNA ratio, did not change during 3 h septicemia or in sham controls where no bacteria were infused (III). The concentration of RNA and DNA was somewhat lower after bacteriemia (29 ± 10 and 184 ± 66 ; $n=8$) compared to basal values (35 ± 14 and 210 ± 77 $\mu\text{g}/100$ mg wet tissue respectively). These values were thus related to tissue wet weight. As judged by the histological findings mucosal edema developed particularly in septic cats. RNA and DNA concentrations were similar in cats given misoprostol and controls (IV). The collagen concentration in the gastric wall related to tissue dry weight did not change during sepsis. It should be stated that the specimens taken for biochemical analysis were obtained from macroscopically normal mucosa not to come in conflict with necrotic tissue.

A general problem in studying the pathogenesis of stress ulcer is the fact that the superficial epithelium, which is only one cell

layer, seems to be the critical point. Probably alterations in the intracellular metabolism and biochemistry of this particular cell layer is difficult to detect by analyzing the whole mucosa.

Prostaglandin. In cats given i.v. misoprostol the gastric mucosal lesion index was 5 or less in 4 of 6 animals (median 4; i.r. 3-7). In sepsis without treatment the lesion index was 6 or more in all cats (median 8, i.r. 7-8; $p < 0.05$). Topical misoprostol resulted in mucosal protection in 3 animals (see fig 2). The lesion index of this series (median 5 i.r. 3-6) was not statistically different from that of controls. However, the number of areas (anterior, posterior wall and greater curvature) with intact superficial epithelium in the different series was significantly less in controls (2 of 24) compared to both i.v. (12 of 18; $p < 0.002$) and i.g. (11 of 18; $p < 0.002$) misoprostol.

Intragastric misoprostol enhanced coeliac artery blood flow which was increased by about 120 % 10 min after drug administration. It subsequently declined to basal during 1 h. Concomitantly, gastric mucosal blood flow increased from 8.4 ± 1.2 to 25.1 ± 9.9 in the fundic and from 6.7 ± 0.8 to 47.8 ± 9.2 ml/min $\times$ 100 g in the antral areas. At late sepsis blood flow was similar to basal. I.v. misoprostol did not affect coeliac arterial or gastric mucosal blood flow.

Local administration of misoprostol was not more effective in gastric mucosal protection than systemic in the present model. However, since we do not know whether the doses given are equipotent concerning "cytoprotective" effects, a strict comparison cannot be made. The doses are equipotent related to inhibition of acid secretion in dogs (29). However, this capacity is probably of no importance in the present experiments since hydrochlorid acid was administered exogenously into the gastric lumen.

Oxygen free radicals. Infusion of SOD and catalase increased the plasma concentration of SOD from 20 ± 1 to 13650 ± 1089 units/ml and of catalase (n=4) from 0 to 4330 ± 718 units/ml. These activities are at the same level as those reported intracellularly (88). Pretreatment with a continuous infusion of oxygen free radical scavengers during live E.coli sepsis resulted in a complete

gastric mucosal protection in 4 of 8 cats while all stomachs in the control group were ulcerated (see fig 2). The median lesion index in the SOD or SOD/catalase group was 5 (i.r. 3-7; $p < 0.1$ vs controls). Significantly more areas with grade 2-4 lesions were found among controls (22 of 24) than in SOD or SOD/catalase treated cats (11 of 24; $p < 0.002$). In contrast, no effect on lesion formation was found in the small intestine (50 % of untreated cats had significant damage (grade 2-5) and 38 % of cats given SOD or SOD/catalase treatment).

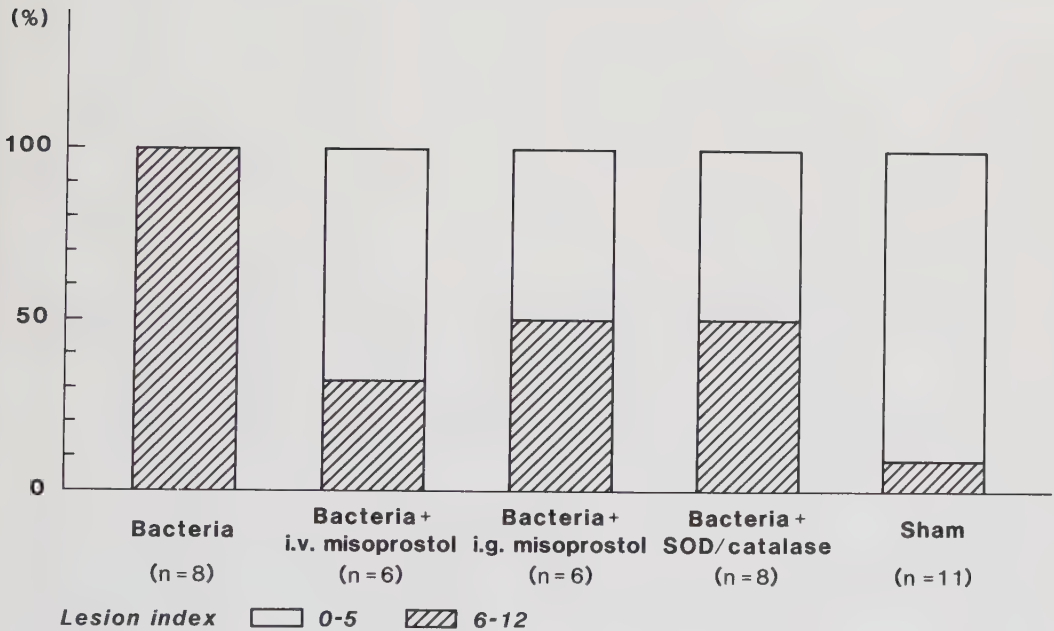


Fig 2. Gastric mucosal damage expressed as % of animals with lesion index 0-5 and 6-12 (III-V). Bile and acid was administered intragastrically in all cats.

GENERAL DISCUSSION

Acute gastric mucosal ulceration is generally considered as a single entity and different forms of stress, such as septic and hemorrhagic shock, are thought to act via the same pathophysiological pathways. This is not necessarily true. According to the general opinion mucosal ischemia is an important, if not obligate prerequisite in the development of stress induced mucosal ulceration (19, 74, 89, 108). This argument is mostly based on experiments utilizing hemorrhagic or regional ischemic models. However, in hemorrhagic shock similar mucosal flow values have been reported in ulcerated and non-ulcerated areas (56). In the present live *E.coli* shock model there was no mucosal ischemia but still mucosal ulceration was always found. It is also reported from other laboratories that gastric or mucosal blood flow is unaltered or even increased upon live *E.coli* bacteriemia (47, 103, 104). Furthermore, in canine live *E.coli* shock decreases in transmucosal potential difference (PD) and intracellular pO_2 of the superficial epithelium occurred simultaneously and immediately after bacterial infusion. This in contrast to hemorrhagic shock where the intracellular oxygen tension decline preceded the PD fall (103). The reaction in septicemia was suggested to be due to reduction in nutrient microcirculatory blood flow. On the other hand, it may also be explained by a direct effect on the superficial mucosal cells.

Redistribution of blood flow to basal parts of the mucosa would be a mechanism by which apical ischemia could occur despite constant total mucosal blood flow. In fact, "functional" arterio-venous shunting has been proposed in canine hemorrhagic shock (14). However, recent microsphere experiments on cats failed to provide support for significant arterio-venous shunts (136) as did microanatomical studies on rats (44).

In the present study live *E.coli* sepsis per se did not induce gastric mucosal ulceration. However, if bile together with 80 mM HCl was administered intragastrically prior to the start of bacterial infusion, mucosal damage regularly occurred during 2 to 3 h of septic state. This is in agreement with previous reports demonstrating acid, regurgitated bile and stress, like hemo-

rrhagic or septic shock, as important pathogenic factors in the development of acute gastric mucosal damage (15, 53, 89, 98, 104, 121). The present series involves a considerable amount of surgical trauma but that alone or combined with topical bile and acid was not sufficient to frequently induce gastric mucosal ulceration.

The pentagastrin-stimulated mucosa was more resistant than non-stimulated mucosa. This is also in agreement with previous reports that pentagastrin- or histamine-stimulated mucosa is less vulnerable (72, 100, 125, 132). It has been argued that this could be an effect of the increased mucosal blood flow or an effect on enhanced alkaline tide, i.e. increased amounts of bicarbonate available as a consequence of hydrogen ion production and secretion (89, 100). Gastric or gastric mucosal blood flow were not measured (II) but it is unlikely that pentagastrin influenced blood flow during septicemia, since the pentagastrin infusion was stopped 15-30 min before the start of E.coli infusion.

In addition to local factors such as acid and bile, systemic acidosis probably contributes a risk factor to the development of mucosal lesions. It has been demonstrated that high plasma levels of HCO_3^- protected the mucosa from ulceration in hemorrhagic shock in rats (67, 126). Similarly, high HCO_3^- levels in perfusate had a protective effect on acid induced lesions in bullfrogs (117). The regeneration capacity was indirectly studied in the present series by the ratio of RNA/DNA. Previously, statistical significant reductions in gastric mucosal DNA synthesis or RNA concentration were found after stress in various species (59, 84, 132) in experiments of 7 to 16 h duration. This time exceeds the experimental period in the present study. However, it could be stated that the results of this study contradict alterations in mucosal RNA or DNA as an initial event of acute mucosal ulceration in septic stress.

Several factors in the mucosal defence system are mediated by prostaglandins which have protective properties on the gastric mucosa subjected to different experimental challenges (see 65, 111). In the present live E.coli shock model misoprostol reduced

the gastric mucosal damage but complete protection was not achieved. The beneficial effects of misoprostol on mucosal integrity have previously been reported in aspirin (25, 28) and aspirin-shock (77) induced damage in dogs as well as stress induced gastric damage in rats (9). Gastric ulceration in peritonitis induced septicemia in dogs was diminished by parenteral 16.16 dimethyl-prostaglandin E_2 (101). The cytoprotective effect of misoprostol and of other prostanoids have been proposed to be mediated through vasodilation (46, 77). This could not be confirmed by the results from this study. I.v. misoprostol, with no effect on blood flow, protected the mucosa at least as well as local application which induced a pronounced increase in mucosal blood flow. Other possible mechanisms behind the cytoprotective effect of i.v. administrated misoprostol include influence on the mucus-bicarbonate barrier. The gastric content of mucus at the end of the experiments was increased in misoprostol-treated animals but not in controls, however, this was not measured objectively. The contribution of the mucus-bicarbonate barrier to the mucosal defence probably does not offer a full explanation since the prostaglandin-stimulated mucus barrier is only capable of withstanding a luminal acid concentration of 10 mM as found in rats (70).

Oxygen derived free radicals are highly reactive and have been proposed to be involved in ischemia and reperfusion induced tissue damage (16). The results of the present study suggest oxygen reduction products as an important factor in the acute gastric lesion formation since only 50 % of the SOD or SOD/catalase pretreated cats had gastric ulcerations but 100 % of the controls. This in contrast to the small intestinal mucosa where similar damage occurred in treated cats and controls (38 and 50 %, respectively).

Cells but not extracellular fluids contain large amounts of enzymes capable to take care of oxygen derived free radicals intracellularly, including superoxide dismutase and catalase (42, 88). By injecting these enzymes i.v. it was possible to achieve a considerable increase in the extracellular activity which reduced the gastric mucosal damage in the present septic model, but no correlation could be found between protection and plasma activ-

ity. In two of the four protected cats SOD alone was sufficient, while in two the combination was infused.

Toxic oxygen reduction products may originate from various sources (42). One of the intracellular enzymes capable of generating superoxide radicals is xanthine oxidase. This enzyme is formed in transient ischemia in the gastrointestinal tract (102, 115) and catalyses the oxidation of hypoxanthine to xanthine, the step prior to the formation of uric acid from purines. It is well established that regional intestinal ischemia (50, 52) and sepsis induced hypotension (36) result in small intestinal mucosal injury. There is no advantageous effects by the inhibition of oxygen free radical generation on the mucosal lesions that develop during ischemia (118). However, after reperfusion the damage is aggravated and this can be abolished or diminished by allopurinol, inhibiting the xanthine oxidase system, or by SOD (102, 118).

Phagocytosing polymorphonuclear leucocytes generate considerable amounts of oxygen derived free radicals (6). These cells are known to be activated in septicemia and septic shock (61). Circulating leucocytes were consumed in the present live *E.coli* shock model and should be considered as a potential source of free radicals.

The gastric mucosa is more resistant to shock than the small intestinal mucosa in that sepsis alone did not induce lesions in the stomach. Sepsis and low pressure perfusion states are known to give hypoxic lesions to the small intestine (1, 36, 50). In the present study mean arterial blood pressure during the last hour of the experimental period was 35 ± 10 (n=7) and 98 ± 11 mmHg (n=12) in animals with and without significant small intestinal mucosal lesions ($p < 0.01$) indicating that hypotension is more important than sepsis per se for the development of small intestinal mucosal damage. A similar relation has previously been described by Falk et al (36). However, gastric mucosal ulcerations developed in all septic cats regardless of blood pressure suggesting that the presence of sepsis is more important than low arterial blood pressure in the pathogenesis of gastric mucosal damage. This difference between the small intestine and the

stomach is explained by the small intestinal counter-current exchange mechanism. The tips of the villi become hypoxic, not because of reduced blood supply but because of low villous flow velocity in hypotension allowing an effective short-circuiting of oxygen at the bases (50, 52, 85). The gastric mucosal vascular anatomy (44) does not provide the prerequisite for a similar mechanism.

CLINICAL IMPLICATIONS

Fully aware that it is hazardous to implicate results from animal experiments to man, I still want to present some reflections.

Gram negative bacteriemia has been stated to be a considerable risk factor in patients developing acute gastric lesions (3, 12, 41, 48, 122). In postoperative and traumatized patients without signs of multiple organ failure septic complications were associated with gastroduodenal mucosal lesions (78). These lesions were found to improve rapidly as soon as the focal infection and septicemia were eradicated (78, 82).

The macroscopical gastric mucosal damage in the present study was located in the fundic area of the stomach. The lesions were multiple, often bleeding and developed during a relatively short period of time. These findings were similar to those seen in the critically ill patient (83). A microscopical examination of the gastric mucosa revealed superficial lesions not penetrating the muscularis mucosae and with a minimal inflammatory reaction (83) - a picture compatible with the histological findings in the present study. Thus it seems justified to consider this experimental model relevant for the clinical stress ulcer situation.

With mortalities exceeding 50 % in patients requiring surgical treatment because of hemorrhage from stress ulcers, the major emphasis has been directed to the possibility of prophylaxis. Apart from increased efforts to improve the general treatment, including the prevention of septic complications and drainage of septic foci, most prophylactic regimens have been directed to the reduction of gastric acidity. This has been accomplished in two different ways - antacids or histamine-2 receptor (H_2) blockade.

The difference between pentagastrin-stimulated gastric mucosa and exogenous supply of acid (II) would suggest antacids instead of H_2 blockade as the optimal mean of prophylaxis. Facing the practical difficulties to achieve neutral intragastric pH in sick patients including the large amounts of antacids needed, a third good alternative would be welcomed. An agent with cytoprotective and acid-secreting inhibitory properties would be appropriate. The beneficial effects of misoprostol in the present study suggest the possibility of using prostanoids for this purpose. Even though the result of a recent clinical study has been rather discouraging (124), further clinical evaluation is desirable.

SUMMARY

- Septic shock by infusion of live E.coli in cats did not induce gastric mucosal damage but small intestinal mucosal lesions developed in about 50 % of the animals.
- Septic shock by infusion of E.coli and, in addition, intragastric administration of 80 mM HCl and 0.6 ml bile/kg regularly induced gastric mucosal ulceration.
- Normotensive sham shock cats (no bacteria) given topical bile and acid did not develop gastric lesions.
- Septic shock by infusion of live E.coli and, in addition, pentagastrin-stimulated gastric acid secretion together with topical bile did not induce lesions.
- Gastric mucosal ulceration occurred regardless of systemic blood pressure, while small intestinal damage was found in hypotensive cats only.
- Septic shock did not change the concentration of RNA, DNA or RNA/DNA ratio in the gastric mucosa.
- There was no difference in gastric mucosal blood flow between the series with and without gastric mucosal damage.

- Misoprostol given i.v. or intragastrically was partially protective against gastric ulceration.
- Topical, but not i.v., misoprostol increased gastric mucosal blood flow.
- Oxygen derived free radical scavengers seemed to protect the gastric mucosa from ulceration.

CONCLUSIONS

Based on the findings in the present study it is concluded

that septic shock, intragastric acid and bile constituted three important factors for the development of acute gastric mucosal ulceration.

that pentagastrin-stimulated acid secretion protected from ulceration.

that the gastric mucosa was more resistant to septic shock than the small intestinal mucosa.

that the development of small intestinal lesions was dependent on low arterial blood pressure, while the gastric mucosa was more vulnerable by bacteriemia per se.

that the gastric lesions did not develop because of decreased mucosal blood flow or decreased mucosal regeneration capacity as measured by RNA/DNA ratio.

that increased gastric mucosal blood flow was not the only mechanism by which misoprostol was protective against lesion formation.

that oxygen derived free radicals were involved in the pathophysiology of the gastric mucosal damage in sepsis.

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0014-312X/84/0164-0220\$2.75/0**Effects of Dihydroergotamine on the Feline Cardiovascular Response to Intravenous Infusion of Live *Escherichia coli* Bacteria**S. Arvidsson^a, B. Lindblad^a, C. Esquivel^a, K. Fält^b, C. Lindström^b, D. Bergqvist^a,
U. Haglund^a^aDepartments of Surgery and ^bPathology, University of Lund, Malmö General Hospital, Malmö, Sweden**Key Words.** *Escherichia coli* bacteremia · Dihydroergotamine pretreatment · Ketanserin pretreatment · Pulmonary vascular reaction · Peripheral blood flow distribution · Gastric mucosa · Intestinal mucosal damage · Countercurrent exchange

Abstract. A septic shock state was induced in cats by intravenous infusion of live *Escherichia coli* bacteria. Cats pretreated with an unspecific 5-HT blocker, dihydroergotamine (DHE), or with a specific 5-HT blocker, ketanserin, were compared with a series receiving bacteria without pretreatment. DHE pretreatment prevented the reduction in systemic arterial blood pressure found in the other series during the 2-hour period of septic shock. Pretreatment could not influence the increased vascular resistance in the pulmonary vascular bed or the early increase in pulmonary arterial blood pressure. Peripheral blood flow distribution was studied using radioactive labelled microspheres. Compared to bacteremia without pretreatment, the 5-HT blockers increased CNS blood flow and ketanserin also prevented the reduction in pancreatic blood flow. Gastric blood flow and gastric mucosal blood flow remained unchanged in all series as did the small intestinal total blood flow. Small intestinal mucosal blood flow, however, was reduced after 2 h of bacteremia. Microscopy revealed no gastric epithelial damage while the jejunal mucosa was characteristically damaged. There was no correlation between the changes in the small intestinal blood flow and the degree of mucosal damage, however, supporting the countercurrent theory for the pathogenesis of these lesions.

Dihydroergotamine (DHE) has a complex effect on the peripheral vascular bed. DHE is an unspecific receptor blocker with effect on alpha-adrenergic and serotonergic receptors. Furthermore, DHE has a partial agonistic effect on the alpha-adrenergic receptors

[17]. The vasoconstriction induced by DHE mainly affects the venous capacitance vessels, while the precapillary resistance vessels are influenced to only a minor extent [15]. DHE, especially combined with low-dose heparin, has been shown to be effective as a

prophylactic measure against postoperative thromboembolism [3, 8]. Therefore, it is possible that DHE will be frequently used in patients undergoing major surgery and trauma, situations which sometimes are complicated with sepsis. It was therefore considered of interest to know to what extent, positively or negatively, treatment with DHE influenced the hemodynamic response to a septic challenge. Therefore, the effect of pretreatment with DHE on the feline response to a standardized bacteremia was investigated. No such studies have been presented. Since DHE is an unspecific receptor blocker the effects were compared to those induced by a specific serotonergic receptor blocker, ketanserin [12, 16].

Material and Methods

The experiments were performed on 18 cats, weighing 2.0–4.5 kg. Anesthesia was induced with pentobarbital (Nembutal®) and continued with chloralose (50 mg/kg body weight) after tracheostomy. The animals were connected to a constant volume respirator and ventilated with 15 ml/kg body weight at 18/min. Thus, the cats were slightly hyperventilated. Dead space was adjusted to give an arterial $p\text{CO}_2$ slightly below normal [7]. End-expiratory and inspiratory pressures in the trachea were recorded through the tracheostomy cannula.

The thorax was opened with a left parasternal thoracotomy, the thymus was dissected away and the pericardium opened. A Swan-Ganz catheter was introduced through the right femoral vein with its tip in the pulmonary artery. The position was checked during thoracotomy and by pressure recordings, and the catheter was used for determining cardiac output by means of thermodilution and for measuring pulmonary arterial blood pressure. A catheter was placed in the left atrium for pressure recordings and for injection of microspheres (see below). Another catheter, used for blood samplings, was placed with its tip in the thoracic aorta via the left femoral artery. Arterial blood pres-

sure was monitored through a catheter in the right femoral artery. Intravenous infusions were given through catheters in the left femoral and brachial veins.

A slow intravenous infusion of a glucose solution, containing bicarbonate (10 mmol NaHCO_3 /100 ml 5.5% glucose; 0.1–0.2 ml/min) was started at the induction of anesthesia and continued throughout the experiments in accordance with earlier reports [10].

Arterial platelet and white blood cell concentrations, hematocrit, pH, $p\text{O}_2$, $p\text{CO}_2$ and oxygen saturation were measured at intervals throughout the experiments.

Bacterial Preparation

An *Escherichia coli* bacteria strain (*E. coli* 06K 13H1) (WHO designation Su 4344/41) from the WHO Collaborative Center for Reference and Research on *Escherichia* (State Serum Institute, Copenhagen, Denmark) was used. The bacteria were cultivated in ordinary nutrient broth. Just prior to infusion the bacteria were washed in saline. The washed bacteria were then resuspended in 0.9% NaCl and infused. In pilot experiments a bacterial concentration of 10^9 live cells/ml was found to induce hypotension within 120 min in most cats and, therefore, this concentration was used.

Determination of Tissue Blood Flow and Distribution

Regional blood flow was determined using the radioactive microsphere technique. The principles and validity of this technique were described in detail by Heymann et al. [11]. A suspension of approximately 900,000 microspheres (diameter $15 \pm 3 \mu\text{m}$, NEN) labelled with ^{141}Ce , ^{103}Ru or ^{46}Sc in 10% dextran containing 0.05% of Tween 80 was vigorously shaken by a mechanical shaker and then immediately injected into the left atrium. The injection time was approximately 30 s. Reference organ flow was taken from the aortic catheter at a rate of 2.22 ml/min and collected 10 s before, during, and 60 s after the injection of the labelled microspheres. The isotopes were given in random order and during the experiment three determinations of tissue blood flow were made.

At the end of the experiment the cats were sacrificed and 60 tissue specimens from each cat were removed and weighed (about 1 g/specimen). The tissue specimens were dissolved in up to 3 ml sulfuric acid and their radioactivity was measured in an auto-

matic gamma-scintillation counter (Ultragamma) equipped with a multichannel analyzer and using suitable windows for optimal discrimination of the isotopes used. The data were further processed according to Heymann et al. [11] to obtain tissue blood flow expressed as $\text{ml}/\text{min}^{-1} \cdot 100 \text{ g}^{-1}$.

Microscopy

At the end of each experiment a piece of the upper portion of the small intestine and the stomach with the proximal part of the duodenum, respectively, was rapidly excised, mounted on cork and fixed in 10% neutral formalin. The blocks were prepared for paraffin embedment, cut at 3–4 μm , mounted and stained with hematoxylin and eosin. The glasses were coded for histological examination and analyzed by the pathologist without any further information on details about the experimental state. The small intestinal mucosal lesions were graded as described earlier [1, 4, 6] into six grades where grade 0 means normal mucosa and grades 1–5 increasing damage to the surface epithelium. Grade 5 is characterized by disintegration of the mucosal lamina propria, hemorrhage, and ulceration.

Experimental Procedures

Following the preparation the animals were allowed to stabilize for about 30 min. Microspheres were then injected in the left atrium. The cats were randomized into three groups where 6 cats received 0.02 mg DHE/kg body weight, 6 cats were given 0.25 mg ketanserin/kg body weight, 4 cats received saline only, and served as controls. The substances were given as a bolus injection 5 min after the microspheres. 5 min later live *E. coli* were infused at a rate of 1 ml/kg body weight $\times$ min for 2 min and then 1 ml/kg body weight $\times$ h for 2 h. Microspheres were injected 3 and 120 min after the start of the bacterial infusion.

In addition, 2 cats were followed throughout the experimental protocol given saline instead of DHE/ketanserin as well as instead of *E. coli* (sham shock).

Statistical Methods

The analysis of the microsphere distribution was performed using a programmable desk computer (Hewlett-Packard) given mean values and standard deviation. Statistical analysis was made according to the nonparametric methods described by Siegel [18]. A *p* level of < 0.05 was considered as significant.

Results

In the sham-shock animals stable conditions without gross changes from the initial level were recorded in all variables throughout the experiments. Tissue blood flow was mainly unchanged with the exception of tissue blood flow to the adrenals which increased threefold.

At the start of the experimental period, mean arterial blood pressure was 124 ± 14 mm Hg in the control series, 106 ± 9 and 128 ± 12 mm Hg in the series later given DHE and ketanserin, respectively. Corresponding values for total peripheral vascular resistance were 1.2 ± 0.1 , 0.9 ± 0.1 , and 1.2 ± 0.1 mm Hg $\times$ min $\times$ kg $\times$ ml^{-1} . DHE induced an increase in arterial blood pressure to $120 \pm 26\%$, and an elevation of total peripheral vascular resistance to $167 \pm 34\%$, while ketanserin reduced blood pressure to $80 \pm 4\%$ with a corresponding fall in peripheral vascular resistance to $82 \pm 5\%$ of the pretreatment value. Upon bacterial infusion there was a rapid reversible pressure reduction in all series, most pronounced in cats without pretreatment (fig. 1). At 2 h the systemic arterial blood pressure was lower in the controls, $63 \pm 21\%$ of the prebacteremic level, than in the pretreated animals. In the DHE series arterial blood pressure remained above the pretreatment level throughout. The peripheral vascular resistance similarly fell during the first minute after bacteremia and then rose rapidly, stabilizing after 15 min. DHE resulted in elevated peripheral resistance persisting throughout the experiment being $137 \pm 14\%$ of the pretreatment value at 2 h (fig. 1).

Cardiac output was 104 ± 14 , 111 ± 6 , and 106 ± 14 ($\text{ml}/\text{min} \times \text{kg}$ body weight) before drug injection in the control, DHE and

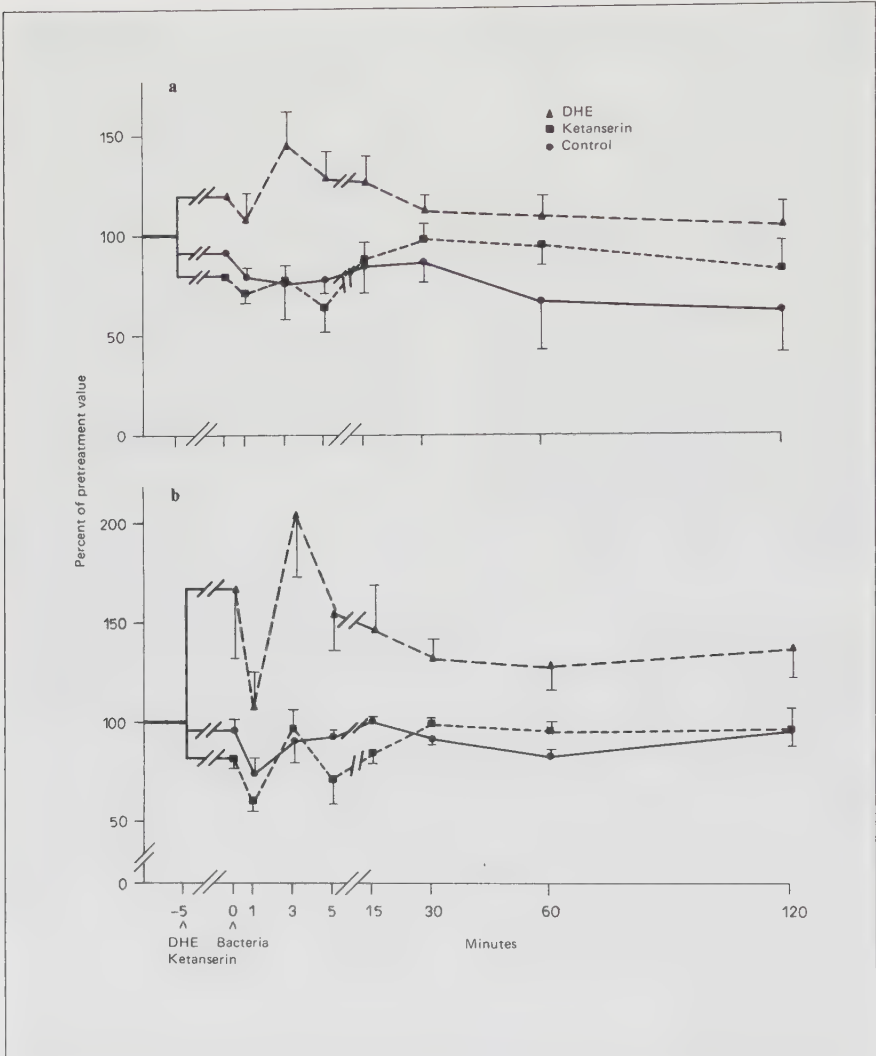


Fig. 1. Mean arterial blood pressure (**a**) and total peripheral resistance (**b**) in untreated ($n = 4$), DHE-pretreated ($n = 6$), and ketanserin-pretreated ($n = 6$) cats following intravenous live *E. coli* infusion. Data expressed as percent of the pretreatment value (mean $\pm$ SE). Symbols representing control cats are connected with a solid line, ketanserin-pretreated cats with a dotted line and DHE-pretreated cats with a broken line.

ketanserin series, respectively. A decrease to $87 \pm 4\%$ of the pretreatment value was seen following DHE, while controls and ketanserin pretreated animals remained unchanged. Cardiac output also responded biphasically during the first 5 min upon bacterial infusion – an initial increase followed by a decrease, and then stabilization at the pretreatment level in ketanserin and control animals, but somewhat lower ($89 \pm 9\%$) in the DHE-pretreated cats. At the end of the 2 h of bacteremia cardiac output was 86 ± 3 , 82 ± 11 , and $83 \pm 11\%$ of the original level in the control, DHE and ketanserin pretreated series, respectively.

Initially, pulmonary arterial blood pressure was 15 ± 2 (mm Hg) in the controls, 18 ± 1 in the DHE and 16 ± 1 in the ketanserin series. It increased by more than 100% with maximal values at 3 min after bacteria. There were no differences between the three groups. A normalization was seen at 15 min persisting throughout the experiment (fig. 2). Neither DHE nor ketanserin could influence the pulmonary vascular resistance which rose to levels of $589 \pm 155\%$ in controls, $394 \pm 145\%$ in DHE and $437 \pm 65\%$ in the ketanserin-pretreated series, respectively (the corresponding initial values were 0.10 ± 0.02 , 0.12 ± 0.01 and 0.15 ± 0.03 mm Hg $\times$ min $\times$ kg $\times$ ml⁻¹). The vascular resistance in the lungs did not normalize after the initial period but returned to a level of about 70% above the pretreatment value without difference between the groups (fig. 2). In all series the maximum inspiratory-expiratory pressure difference in the trachea was increased about 50% upon bacterial infusion. After 15 min of bacteremia this pressure difference returned to the baseline level.

The number of white blood cells in arterial blood decreased drastically upon bacte-

rial infusion and remained low also at 60 and 120 min. The polymorphonuclear cells decreased from about 70% ($6.6 \pm 1.2 \times 10^9$ cells/l) to 4–14% of the total number of leucocytes with no statistical difference between the series. The mononuclear white blood cells remained roughly unchanged during the experiments ($2.2 \pm 0.3 \times 10^9$ cells/l). The platelets decreased 58–67% 5 min after bacteremia compared to the pretreatment level ($239 \pm 30 \times 10^9$ cells/l). The number of platelets was almost normalized at 2 h. Again there was no difference between the series. Arterial pO₂ was 14.8 ± 0.7 , pCO₂ 3.6 ± 0.3 (kPa), and pH 7.44 ± 0.02 before bacterial infusion without any difference between the series. No significant changes were seen during the experiments, while pH was reduced to 7.40 ± 0.02 at 2 h in all groups.

The distribution of microspheres indicated increased blood flow to the CNS after 3 and 120 min of bacteremia in DHE- and after 120 min in ketanserin-pretreated animals (table I). The adrenal glands had a flow at 2 h of bacteremia of about three times the pretreatment level with no differences between the groups. Kidney blood flow was increased at 3 and 120 min in the DHE-pretreated cats, and a tendency to increase was also seen at 120 min in the controls. Pancreatic blood flow was reduced in the control and in the DHE series while it was maintained in the ketanserin-pretreated cats. No significant changes in blood flow were seen in the myocardium, the liver or the colon. No significant difference between right and left kidney tissue blood flow was seen (range 0–17%).

The gastric (fundic) and small intestinal (jejunal) blood flow were subjected to special analysis (table II). Total as well as mucosal gastric blood flow was well maintained

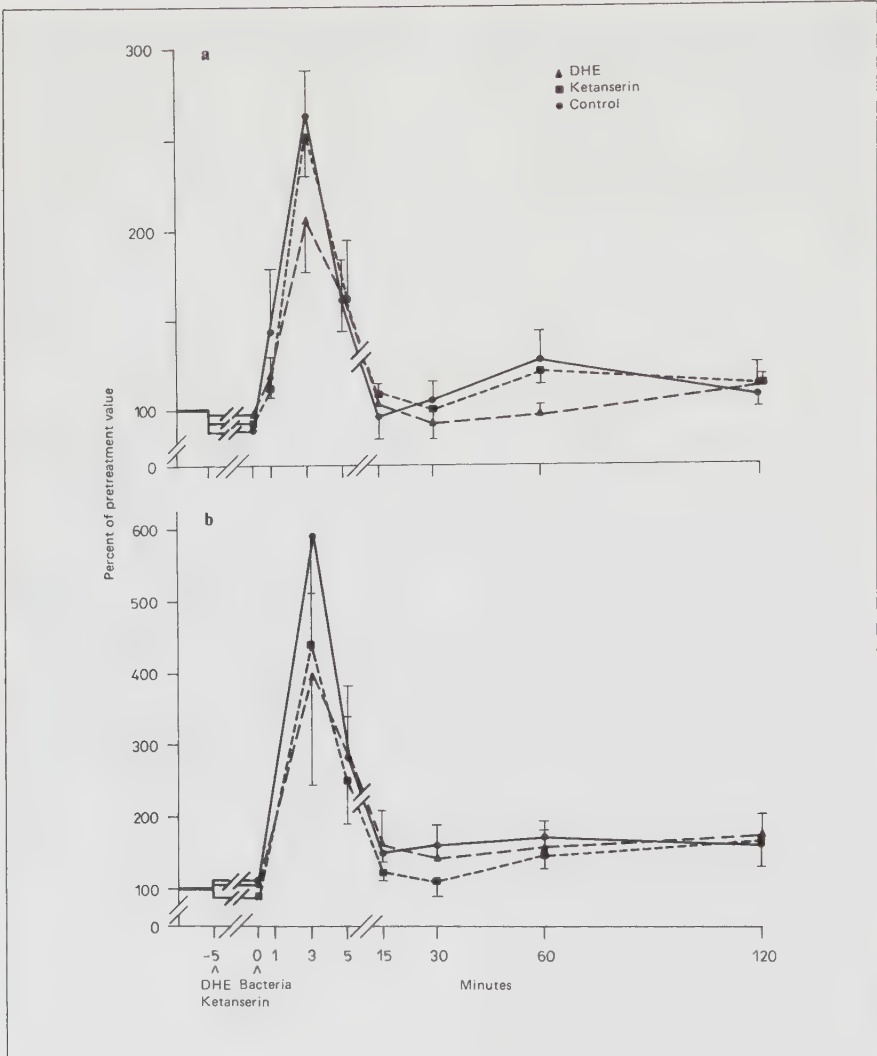


Fig. 2. Pulmonary arterial blood pressure (a) and pulmonary vascular resistance (b) in untreated ($n = 4$), DHE-pretreated ($n = 6$), and ketanserin pretreated ($n = 6$) cats following intravenous infusion of live *E. coli*. Mean $\pm$ SE. Symbols representing control cats are connected with a solid line, ketanserin-pretreated cats with a dotted line and DHE-pretreated cats with a broken line.

throughout the septic shock. In table III the corresponding variables for the small intestine are given. Total intestinal blood flow was also well maintained in the prebacteremic range throughout the experiments. Intestinal mucosal blood flow, however, was reduced at 120 min following bacterial infusion ($p < 0.05$).

The microscopic examination revealed no damage in the gastric mucosa. The gastric epithelium was intact in all specimens (fig. 3). In the small intestinal mucosa, however, characteristic hypoxic lesions were seen in all specimens (table IV; fig. 4). In 14 of these 16 animals, data on blood flow distribution within the intestinal wall is available.

Table I. Tissue blood flow (ml/min $\times$ 100 g)

		Pretreatment	Time after inducing bacteremia	
			3 min	120 min
Brain, n = 8 (cerebrum, cerebellum, thalamus, medulla oblongata)	C	16 $\pm$ 4	17 $\pm$ 3	14 $\pm$ 2
	D	12 $\pm$ 3	15 $\pm$ 3	16 $\pm$ 3
	K	14 $\pm$ 3	15 $\pm$ 3	20 $\pm$ 5
Myocardium, n = 4 (left and right ventricle)	C	147 $\pm$ 45	135 $\pm$ 51	93 $\pm$ 20
	D	66 $\pm$ 22	86 $\pm$ 16	63 $\pm$ 17
	K	97 $\pm$ 16	87 $\pm$ 14	107 $\pm$ 26
Kidney, n = 2 (cortex and medulla)	C	67 $\pm$ 10	69 $\pm$ 12	102 $\pm$ 11
	D	51 $\pm$ 11	66 $\pm$ 16	75 $\pm$ 19
	K	55 $\pm$ 10	63 $\pm$ 11	59 $\pm$ 17
Liver, n = 2 (blood flow in the hepatic artery)	C	19 $\pm$ 5	17 $\pm$ 4	15 $\pm$ 5
	D	18 $\pm$ 6	20 $\pm$ 7	24 $\pm$ 7
	K	25 $\pm$ 10	18 $\pm$ 6	11 $\pm$ 4
Pancreas, n = 2 (cauda and corpus)	C	17 $\pm$ 6	8 $\pm$ 2	8 $\pm$ 3
	D	17 $\pm$ 3	26 $\pm$ 4*	9 $\pm$ 1*
	K	20 $\pm$ 5	22 $\pm$ 5	16 $\pm$ 4
Colon, n = 2 (proximal and distal)	C	34 $\pm$ 18	26 $\pm$ 15	23 $\pm$ 11
	D	17 $\pm$ 3	20 $\pm$ 4	17 $\pm$ 4
	K	21 $\pm$ 6	16 $\pm$ 5	17 $\pm$ 6
Adrenal glands, n = 2	C	194 $\pm$ 26	186 $\pm$ 66	745 $\pm$ 173
	D	143 $\pm$ 27	192 $\pm$ 52	429 $\pm$ 81*
	K	144 $\pm$ 28	109 $\pm$ 18	455 $\pm$ 133*

Mean values $\pm$ SE; * Denotes statistical significance compared to the pretreatment value. C = Control (untreated) series (4 cats); D = DHE-pretreated series (6 cats); K = ketanserin-pretreated series (6 cats).

n = Number of specimens analyzed from each tissue. In the further processing of data the mean value from each animal was used.

In the cats showing a mucosal damage judged as grade I-II mucosal blood flow at 120 min after bacteremia was 8.4 ± 1.6 ($n = 6$). The corresponding value for the cats with grade III-V damage was 17.1 ± 6.3 ml/min $\times$ 100 g ($n = 8$). This difference was not statisti-

cally significant. There was no correlation between the degree of mucosal damage and the intestinal mucosal blood flow at 120 min of bacteremia ($r = 0.44$; $p > 0.05$), nor was there any correlation between mucosal damage and the type of pretreatment given.

Table II. Gastric blood flow (ml/min $\times$ 100 g)

		Pretreatment	Time after inducing bacteremia	
			3 min	120 min
Total	Control (4 cats)	8.1 ± 1.9	5.7 ± 1.5	6.3 ± 1.3
	DHE (5 cats)	5.1 ± 1.1	6.6 ± 1.6	4.7 ± 1.2
	Ketanserin (5 cats)	8.8 ± 3.2	7.8 ± 2.2	10.8 ± 5.1
	Σ (14 cats)	7.3 ± 1.3	6.8 ± 1.0	7.4 ± 2.1
Mucosal	Control (4 cats)	15.1 ± 7.0	9.7 ± 5.0	12.4 ± 5.8
	DHE (5 cats)	5.7 ± 0.9	9.3 ± 3.9	7.8 ± 2.2
	Ketanserin (5 cats)	17.1 ± 6.1	15.5 ± 2.7	21.2 ± 7.0
	Σ (14 cats)	12.5 ± 3.1	11.6 ± 2.2	14.0 ± 3.3
Mean values ± SE.				

Table III. Small intestinal blood flow (ml/min $\times$ 100 g)

		Pretreatment	Time after inducing bacteremia	
			3 min	120 min
Total	Control (4 cats)	17.5 ± 4.1	13.3 ± 2.8	14.2 ± 2.5
	DHE (5 cats)	11.4 ± 2.2	16.5 ± 2.4	13.0 ± 1.7
	Ketanserin (5 cats)	16.0 ± 3.4	13.6 ± 2.3	22.1 ± 6.8
	Σ (14 cats)	14.8 ± 1.8	14.5 ± 1.4	16.8 ± 2.9
Mucosal	Control (4 cats)	21.0 ± 3.6	17.3 ± 3.1	7.8 ± 0.3
	DHE (5 cats)	20.0 ± 3.9	25.1 ± 2.9	11.5 ± 2.9*
	Ketanserin (5 cats)	25.1 ± 6.9	22.7 ± 5.0	17.9 ± 9.0
	Σ (14 cats)	22.3 ± 3.0	22.1 ± 2.2	13.1 ± 3.6*

Mean values $\pm$ SE; * denotes statistical significance compared to the pretreatment value.

Two specimens were analyzed from each animal and the mean was chosen for further processing of data.

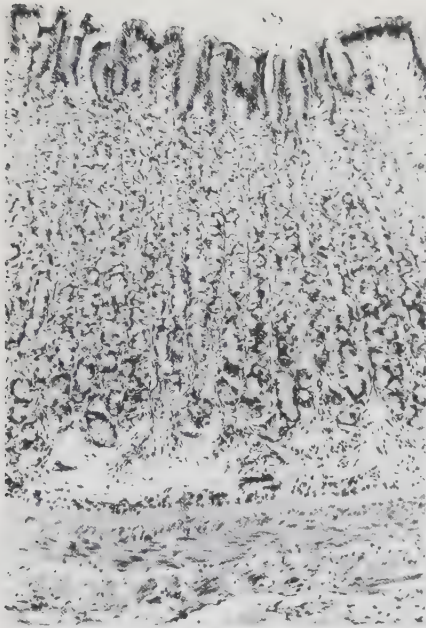


Fig. 3. Gastric mucosa with normal surface epithelium. No signs of ischemic necrosis. HE. $\times 23$.

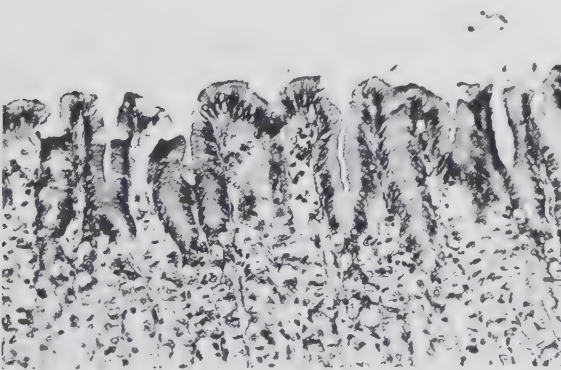


Fig. 4. Gastric mucosa with normal surface epithelium. No signs of ischemic necrosis. Detail of the surface epithelium. HE. $\times 55$.

Discussion

In the present study it was found that pretreatment with DHE, an unspecific receptor blocker with alpha-adrenergic and serotonergic receptor affinity effects [17] prevented the general hypotension induced in unpretreated cats subjected to standardized bacteremia. Ketanserin, a specific serotonergic-2 receptor blocker [12, 16] was not equally effective in preventing the development of hypotension. Neither did DHE nor ketanserin influence the rise in pulmonary arterial pressure induced by bacterial infusion or the increase in pulmonary vascular resistance, the decrease in the number of circulating arterial leukocytes or platelets. This lack of

Table IV. Grade of damage in the small intestinal mucosa

	Grade					
	0	1	2	3	4	5
Number of animals	—	3	5	4	3	1

influence on the response to the standardized bacteremia [5] has for ketanserin been demonstrated earlier in a similar experimental model [2].

In the control series subjected to bacteremia, cardiac output was reduced to $86 \pm 3\%$ of control. The pancreatic blood flow was reduced. Blood flow to the adrenal glands was increased as also seen in the sham experiments. Compared to the control series, the DHE- and ketanserin-pretreated series showed a similar pattern of reactions except that the cerebral blood flow was increased in these two groups (table I).

Among the other vascular beds it is of certain interest to notice that both DHE and ketanserin increased the cerebral blood flow during bacteremia (table I). It has been shown in dogs that DHE increases the blood flow to the CNS [13]. The mechanism behind this effect on this specific vascular bed is not clear.

Gastric and small intestinal blood flow was increased in ketanserin-pretreated animals at 120 min of bacteremia. The difference in tissue blood flow distribution reflects,



Fig. 5. Jejunal mucosa with marked ischemic degeneration and necrosis of the surface epithelium leading to total denudation of the villi (grade 4). HE. $\times 23$.

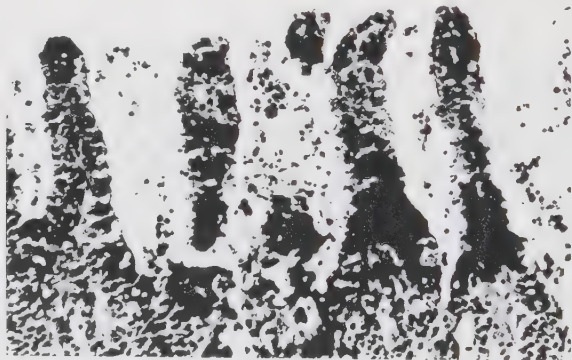


Fig. 6. Jejunal mucosa with marked ischemic degeneration and necrosis of the surface epithelium leading to total denudation of the villi (grade 4). Detail of the villi. HE. $\times 55$.

since cardiac output was reduced in the same order of magnitude in all series, differentiated actions on different organ vascular smooth muscles.

The total gastric fundic blood flow as well as fundic mucosal blood flow remained in the prebacteremic range throughout the experimental period in all series. Corresponding data from the small intestine revealed a maintained total intestinal blood flow but reduced intestinal mucosal blood flow late in bacteremia. The microscopic examination of the gastric mucosa revealed no significant changes induced by the bacteremia. In the small intestine, on the other hand, typical mucosal damage was found. It is, however, unlikely that the difference in the behavior between the gastric and the intestinal mucosa is explained by the fact that the blood flow to the intestinal mucosa was reduced late during bacteremia. Thus, it was demonstrated that intestinal mucosal blood flow was more reduced in the cats with discrete mucosal damage while it was rather maintained in the prebacteremic range in those cats having pronounced intestinal mucosal damage. The lack of correlation between the degree of intestinal mucosal damage and blood flow also indicates that other mechanisms than mucosal ischemia are responsible for the development of the mucosal damage. On the other hand, it has been demonstrated that the mucosal damage is caused by hypoxia [1, 9]. This rather paradoxical situation could be explained by the intestinal counter-current mechanisms [1, 9, 14]. According to this hypothesis the tip of the villus becomes hypoxic, not because of reduced blood supply but because of reduced blood flow velocity in the villus. The reduced flow velocity will allow a more effective short-circuiting of oxygen in the base of the villus. The vascular anatomy

in the villus implies a central arterial vessel and a subepithelial network of capillaries and venules. The distance between the arterioli and the venules/capillaries is less than 20 μm which will allow cross-diffusion of easily diffusible substances provided there is a concentration difference. Such is the case for oxygen, and an equilibrium between arterial and venous blood could more easily be developed in a situation with reduced flow velocity [14].

In conclusion, it seems clear that DHE gives no clear-cut advantages in septic shock states, but also that DHE treatment does not induce any unfavorable reactions. The specific serotonergic receptor blocker, ketanserin, had no significant other effect than DHE. Furthermore, it was demonstrated that the small intestinal mucosal lesions do not develop in septic states because of insufficient blood supply to the mucosa. This finding provides additional support for the counter-current hypothesis for the pathogenesis of these lesions.

Acknowledgements

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Acute gastric ulceration in septic shock: an experimental model

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INTRODUCTION

Bleeding acute lesions in the gastric mucosa not infrequently complicate stress situations such as trauma, haemorrhage and septic shock (1). The mucosal damage is generally the result of an imbalance between the protective properties of the mucosa and different destructive agents in the lumen such as hydrochloric acid and regurgitated duodenal contents. It appears that the combined topical effects of bile and hydrochloric acid on the gastric mucosa on one hand and a stress (shock) situation on the other are important in initiating gastric lesions (9). However, the necessity of the presence of *all* three factors for ulcerations to develop is still unresolved. The exact mechanisms behind the development of acute gastric mucosal damage in response to stress remain unclear. Different experimental models have been developed to study the aetiology and pathophysiology (4, 10, 11, 15). The aim of this study was to find a septic shock model where gastric damage regularly develops and which could be used for future studies on pathogenesis.

MATERIAL AND METHODS

Eighteen cats (2.6–4.6 kg) were deprived of food for 24 hours but had free access to water. Anaesthesia was induced with pentobarbital (Pentothal® approximately 50 mg i.v.) and continued with chloralose (50 mg/kg) after tracheostomy. The animals were connected to a constant volume respirator and ventilated with room air (15 ml/kg at 18/min) as described previously (2).

OPERATIVE PROCEDURES

A thoracotomy was performed and the pericardium was cut open. Pulmonary artery pressure was recorded by a Swan-Ganz catheter (Edward®, 5F), positioned in the pulmonary artery via the right femoral vein or by a catheter introduced

directly into the pulmonary artery and kept in place by a purse string suture. Arterial blood pressure was monitored from the right femoral artery. Intravenous infusions were given in the left brachial and femoral veins. The cats were laparotomized and splenectomized. A tube was placed in the stomach through a duodenotomy and kept in place by means of a tight ligature around the pylorus. It was connected to a saline reservoir placed 2 cm above the pylorus, thus ensuring a constant intragastric pressure. A slow i.v. infusion of glucose solution, containing bicarbonate (10 mM. NaHCO₃/100 ml 5.5% glucose; 0.1–0.2 ml/min) was started at the induction of anaesthesia and continued throughout to maintain arterial pH at a normal level (6).

Bacterial preparation: An *Escherichia coli* strain (*E. coli* 06K 13H1) (WHO designation Su 4344/41) from the WHO Collaborative Centre for Reference and Research on *Escherichia* (State Serum Institute, Copenhagen, Denmark) was used. The bacteria were cultivated in ordinary nutrient broth. Immediately prior to infusion the bacteria were washed in saline, resuspended in 0.9% NaCl and infused at a concentration of 10⁹ live cells/ml. All cats received 1 ml/kg/min for 2 min and then 1 ml/kg/hour for up to three hours.

EXPERIMENTAL PROCEDURES

Following the preparation the animals were allowed to stabilize for 30 minutes. All animals had bacteria infused as described above and were divided into four groups. Group 1 (n=2) received bacteria only. In group 2 (n=4) the cats also had 0.6 ml of gallbladder bile/kg instilled into the stomach one minute before the start of bacterial infusion. Cats in group 3 (n=6) had an i.v. continuous infusion of pentagastrin (Peptavlon®), 16 µg/kg during the 45 minutes just preceding the induction of bacteraemia and also bile as above. In group 4 (n=7) bile as well as 80 mM. HCl was instilled intragastrically one minute before the bacteria.

After 150 minutes of bacterial infusion the experiments were terminated and the stomach rapidly excised for macroscopical evaluation of

gastric mucosal damage. No microscopic examination was performed.

STATISTICAL METHODS

Data are expressed as mean value $\pm$ SE if based on five or more observations; otherwise the mean value alone is given.

RESULTS (TABLE I)

Macroscopical gastric mucosal lesions were only seen after intragastric instillation of bile and HCl (group 4). In the three other groups no macroscopical mucosal damage developed although most animals reacted with a profound hypotension towards the end of the experiments. Arterial blood pressure decreased from a pre-septic value of 110 ± 8 to 40 ± 12 mmHg in groups 1–3 and 43 ± 17 mmHg in group 4 at 150 minutes. Pulmonary artery blood pressure increased uniformly in all groups to a level of about 2.5 times the pre-septic value upon bacterial infusion but was normalized after 15 minutes. Intra-gastric pH was about 1.0 in the pentagastrin stimulated (range 0.9–1.0) as well as in cats given 80mM HCl i.g. (range 1.1–1.2) before the instillation of bile.

Table I. Macroscopical gastric mucosal lesions in septic shock

	n	Number of stomachs with gastric mucosal lesion
Bacteria	2	0
Bacteria and bile	4	0
Bacteria and bile and Peptavlon®	6	0
Bacteria and bile and 80mM HCL	7	7

All lesions were located in the gastric body while the antral area appeared to be normal. The damage showed patterns of long fissures or larger areas with superficial erosions.

DISCUSSION

In this study the animals were challenged with the combination of significant surgical trauma and bacteraemia. This trauma, alone or combined with intragastric bile and/or pentagastrin-

stimulated acid secretion, did not induce gastric mucosal lesions. However, topical administration of 80mM. HCl and bile together with i.v. bacterial infusion regularly induced gastric mucosal damage. Septicaemia alone has earlier been shown not to induce gastric lesions while the small intestinal mucosa was regularly affected (2). Furthermore, this is in agreement with earlier reports that acid and bile together with a stress factor are necessary to induce gastric lesions (4, 9, 12).

Factors involved in the formation of acute gastric ulcerations have been explored experimentally during the last decade. The problem is generally looked upon as an imbalance between protective and aggressive mechanisms acting on the gastric mucosa. The term "gastric mucosal barrier" has been proposed as a collective name of the mucosal defence mechanisms (5). Nevertheless, recent data indicate that at least partly the defence is located outside the mucosa in the mucus layer (17). The development of gastric mucosal damage is influenced by "barrier breakers" such as bile (7) which promotes increased back diffusion of H^+ , resulting in tissue acidosis and damage (9). On the other hand, back diffusion of H^+ did not induce damage to the mucosa unless combined with mucosal ischaemia (12). The result of this study therefore supports the view that all three factors – barrier breaker (bile), stress (surgery and sepsis) and acid – are important parts in the pathogenesis of the gastric mucosal lesions.

The difference in ulcerogenicity between pentagastrin-stimulated gastric acidity and intragastric instillation of HCl is intriguing. Pentagastrin was given during the 45 minutes preceding the bacteraemia to avoid differences in acid secretory response due to differences in mucosal blood flow responses. Pentagastrin increases gastric mucosal blood flow (13) but this effect is unlikely to affect the chain of events during the bacteraemic period. The difference in effect resulting from HCl placed into the stomach and pentagastrin-stimulated HCl secretion might be explained by a trophic influence of pentagastrin on the gastric mucosa resulting in less decrease in DNA synthesis and cell turnover as proposed by Takeuchi & Johnson (16). Increased mucosal tide of HCO_3^- concomitant with stimulated acid secretion resulting in better buffering capacity to back diffusing H^+ is discussed by Kivilaakso & Silen (9). This theory is also favoured by the finding that mucosa which is

actively secreting acid is more resistant to ulceration than is the resting or normal mucosa (14). Intramural pH has been shown to rise to levels of 8 in stimulated as compared to pH 7.3 in the resting gastric gland, which is in agreement with the above theory (8). In contrast Bakke et al. (3) found that mucosal erosions developed in haemorrhagic shock in combination with pentagastrin infusion prior to haemorrhage. However, in our model, lesions could not be seen by macroscopic examination in cats given pentagastrin and microscopical evaluation of the mucosa was not performed.

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ACUTE GASTRIC MUCOSAL ULCERATION IN SEPTIC SHOCK

An Experimental Study on Pathogenic Mechanisms

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Abstract. Intravenous infusion of live, *E. coli* bacteria in cats did not induce microscopic damage to the gastric mucosa within 3 hours. However, if the cats before the induction of bacteremia were given 80 mM HCl and 0.6 ml gallbladder bile/kg b.w. microscopic mucosal damage developed regularly in the corpus-fundus area of the stomach. The gastric mucosal damage was not associated with significant decrease of total gastric blood flow as measured continuously electromagnetically or decreased gastric mucosal blood flow measured early and late during sepsis using radioactively labelled microspheres. Neither was the development of gastric mucosal damage associated with reduced gastric wall collagen concentration nor in RNA, DNA concentration or RNA/DNA ratio in the gastric mucosa.

Acute bleeding lesions in the gastric mucosa are considered to be a component in multiple organ failure complicating severe trauma, sepsis and septic shock (4, 20). The exact pathogenesis of acute gastric mucosal damage in response to severe stress remains unclear. Generally speaking, the mucosal damage is caused by imbalance between protective properties of the mucosa and destructive factors in the lumen. Important factors are hydrochloric acid and regurgitated bile and pancreatic juice (13, 26). Among the mechanisms that have been discussed as causing impairment of protection are mucosal ischaemia (3, 18), decreased mucus (22) and bicarbonate (31) secretion, and reduction of epithelial cell turnover and protein synthesis (15).

The present study was performed with a standardized experimental model (1), with the aim of observing possible pathogenetic mechanisms of acute gastric ulceration in the septic cat. The development of acute mucosal damage thus was studied in relation to gastric mucosal blood flow, mucosal RNA/DNA ratio changes and collagen concentration.

MATERIALS AND METHODS

The experiments were performed on 19 cats weighing 2.6-4.6 kg. They were deprived of food for 24 hours but had free access to water. Anaesthesia was induced with approximately 50 mg pentobarbital (Pentothal®) and continued with chloralose (50 mg/kg bw) after tracheostomy. The cats were connected to a constant volume respirator and ventilated with room air (15 ml/kg bw at 18/min) as previously described (2).

Operative procedure

Thoracotomy was performed and the pericardium was cut open. A Swan-Ganz catheter (Edward®, 5 F) was positioned in the pulmonary artery via the right femoral vein, connected to an Edward® computer (1520) and used to measure cardiac output (CO)—thermodilution; 2 ml 5.5% glucose at 0-1°C—and pulmonary arterial blood pressure (PAP). A blood sampling catheter was inserted into the thoracic aorta via the left femoral artery. Arterial blood pressure was monitored from the right femoral artery. The left brachial and femoral veins were used for infusions. The cats were laparotomized and splenectomized. A tube was placed in the stomach through a duodenotomy and was kept in place by means of a tight ligature around the pylorus. It was connected to a saline reservoir placed 2 cm above the pylorus, thus ensuring a constant intragastric pressure. A slow intravenous infusion of glucose containing bicarbonate (10 mmol NaHCO₃/100 ml 5.5% glucose; 0.1-0.2 ml/min) was started at the induction of anaesthesia and continued throughout to maintain arterial pH at a normal level (12).

A polyethylene catheter was positioned in the left atrial auricle for injection of radioactively labelled microspheres. An electromagnetic flow probe (Cliniflow model 601 D, Carolina Medical Electronics, USA) was placed on the coeliac axis for approximate measurement of gastric blood flow. The hepatic artery was ligated proximal to the gastroduodenal artery, allowing collateral circulation to the liver.

Determination of gastric mucosal blood flow

A suspension of approximately 900 000 microspheres (diameter 15±3 µm, NEN) labelled with ¹⁴¹Ce, ¹⁰³Ru or ⁴⁶Sc in 10% Dextran containing 0.05% of Tween 80 was

vigorously shaken by a mechanical shaker and injected in random order into the left atrium during 15 sec. This was performed before and 15 and 150 min after the start of bacterial infusion. However, if the arterial blood pressure fell to a level of ≤ 50 mmHg, the last injection was made at that time (range 70–150 min). The times for injection are therefore referred to as "early" and "late" sepsis. Data from all three measurements were obtained only from eight control and eight bacteraemic cats. A reference blood sample was withdrawn from the aorta at a speed of 3.4 ml/min, starting 10 sec before the microsphere injection and continued for 70 sec. At the ends of the experiments, parts of the fundus, the major curvature and the antrum were excised and the mucosa was dissected free and dissolved in 3 ml concentrated sulphuric acid. The radioactivity was measured in an automatic gamma-scintillation counter (Ultragamma®). The gastric mucosal blood flow was calculated with the formula:

$$\text{Tissue blood flow (ml/min} \times 100 \text{ g tissue)} = \frac{\text{tissue radioactivity} \times \text{reference flow}}{\text{reference radioactivity}}$$

Histologic examination

After removal of the specimens for gamma-counting and biochemistry (see below), the remainder of the stomach was mounted on cork and fixed in 4% buffered formalin. The blocks were prepared for paraffin embedding, cut at 3–4 μm , mounted and stained with haematoxylin-eosin. The slides were coded for histologic examination. They were analyzed by the pathologist without further information on details concerning the experimental state. The microscopic examination was made from predetermined sites of the anterior and the posterior wall and the major curvature. The mucosal lesions were graded 0–4: grade 0=normal mucosa (Fig 1), grade 1=oedema just beneath the superficial epithelium, grade 2=disappearance of the surface epithelial cells, grade 3=damage to the upper half of the glandular cells of gastric crypts, and grade 4=disappearance of the glands. The gastric mucosal damage index was calculated as the sum of the maximum damage in the three fundic areas. The gastric damage index obtained in the individual animals thus ranged from 0 to 12.

Biochemical analyses

A. Collagen determination. A small biopsy specimen was extirpated from the anterior wall of the gastric body before induction of bacteraemia and was analyzed for collagen. At the end of the experiments, two additional biopsies of total gastric wall (5 $\times$ 5 mm) were made, from the posterior wall and the major curvature. All specimens were dried in an oven at 100°C to constant weight. Collagen was determined as hydroxyproline after hydrolysis (6 N HCl for 3 hours at 130°C) as previously described (24, 29). The hydroxyproline concentration was expressed as $\mu\text{g/mg}$ dry tissue.

B. RNA/DNA content. Measurement of RNA (7) and DNA (6) was made in 5 $\times$ 5 mm pieces of the gastric mucosa taken at the same times and from the same areas as the collagen biopsies. Standards were obtained from Sigma Chemical Company, St Louis, USA.

Bacterial preparation. An *Escherichia coli* strain (*E. coli* 06K 13H 1) was used in the experiments. The strain (WHO designation Su 4344/41) was obtained from the WHO Collaborative Centre for reference and research on *Escherichia* (State Serum Institute, Copenhagen, Denmark). The bacteria were cultivated in ordinary nutrient broth and just before infusion they were washed in physiologic saline solution. After resuspension in 0.9% NaCl the bacteria were infused in a concentration of 10^9 live cells/ml. The cats received 1 ml/kg bw $\times$ min for 2 min and then 1 ml/kg bw $\times$ hour for periods up to 3 hours.

Experimental procedure

All cats received intragastric instillation of 0.6 ml gallbladder bile/kg bw and 80 mM HCl one min before intravenous infusion of *E. coli* ($n=8$) or, in control cats, saline ($n=11$). Arterial blood was sampled for determination of platelet and white blood cell concentrations and pH, pO_2 and pCO_2 before, and at early and late sepsis.

Statistical methods

Data are expressed as mean value $\pm$ SE if based on five or more observations; otherwise the mean value alone is given. Ordinary scale data, however, are given as median and interquartile range (i.r.). When testing statistical significance and correlation, nonparametric methods (27) were used. A p -level of <0.05 was considered as significant.

RESULTS

Haemodynamics. The mean systemic arterial blood pressure decreased from 130 ± 9 mmHg to 38 ± 23 at 150 min in the septic group and from 129 ± 6 to 87 ± 16 mmHg in the controls. The median survival time was 140 min (range 80–180) and 180 min (range 115–180), respectively (NS). PAP was 14 ± 1 and 15 ± 2 mmHg in the two groups. It increased to 34 ± 4 at 3 min in the cats infused with bacteria but remained unchanged (16 ± 2) in the controls. PAP had normalized 15 min after the infusion (16 ± 2 and 15 ± 2). Pressure levels at the end of the experiments were, respectively, 21 ± 3 and 18 ± 3 mmHg.

CO in the prebacteraemic period was 313 ± 31 ml/min in the cats which later received *E. coli* and 361 ± 26 ml/min in the control group (NS). CO rose in the control group during the first min of infusion (Fig. 2). At the end of the experiments the respective CO values had fallen to 197 ± 43 and 200 ± 11 ml/min. The respective group values for prebacteraemic blood flow in the coeliac axis were 34 ± 8 , 39 ± 5 ml/min. The flow remained virtually constant in the septic cats. Saline infusion (controls) induced a slight increase during the first minutes, but in these cats there was significant reduction of gastric

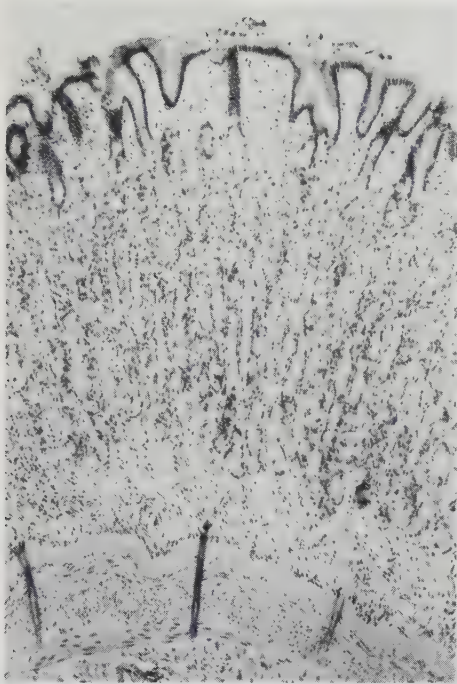
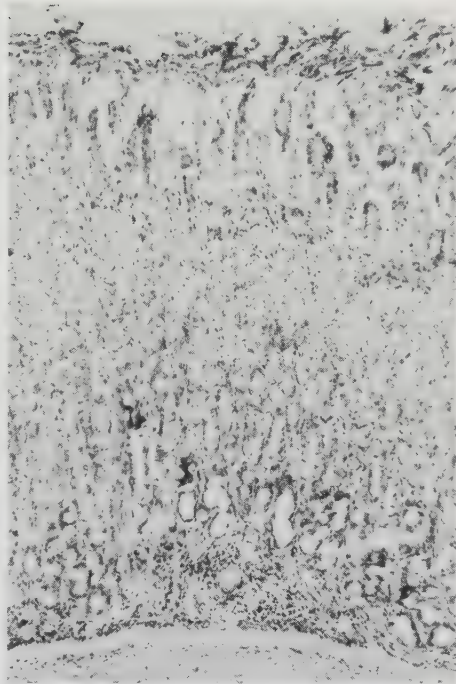


Fig. 1. Representative photomicrographs illustrating the grading system used for gastric mucosal lesions. (a) A grade 1 lesion characterized by subepithelial oedema. (b)



A grade 2 lesion with typical loss of the surface epithelium. (c) A grade 4 lesion characterized by loss of most of the glandular tissue.

blood flow during the last 90 min (Fig. 2). Gastric mucosal blood flow in the corpus (Fig. 3) is presented as the mean of values from corpus and major curvature areas. There was no significant difference between antral and corpus flows, or between the control group and the bacteraemic group. Mucosal blood flow remained within the pre-septic range in early and in late sepsis.

Histologic examinations. The index of gastric mucosal damage (Table I) was significantly higher in the bacteraemic cats (median 8; i.r. 7–8) than in the controls (median 3; i.r. 2–4; $p < 0.01$). All the cats in the septic group had grade 3 or 4 lesions, except for one with grade 2 at all the examined sites. All the bacteraemic animals thus had disruption of the superficial epithelium. Among the 11 controls, 7 had grade 0–1 lesions only, i.e. had intact superficial epithelial lining.

Biochemical analyses. The collagen concentra-



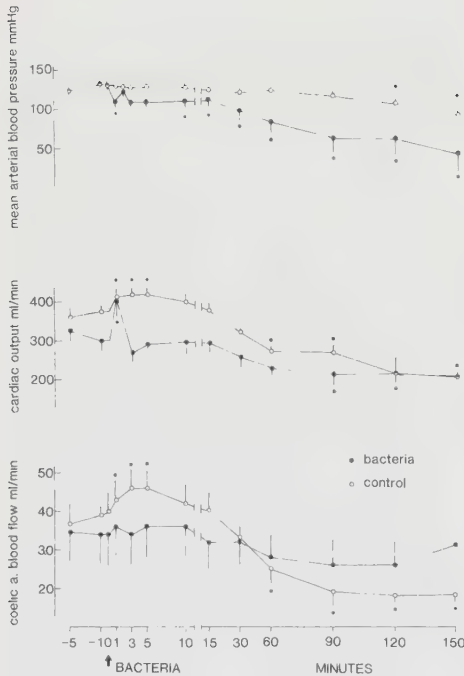


Fig. 2. Mean arterial blood pressure, cardiac output and coeliac artery blood flow in cats with induced bacteraemia ● and control cats ○. * denotes significant difference ($p < 0.05$) compared with basal value.

tion in the total gastric wall biopsies before infusion was similar in the two groups of cats (Table II). The postinfusion (bacteria/saline) values for collagen are stated as the mean in the specimens taken from the corpus of the stomach. There was no significant change in collagen concentration after 3 hours of bacteraemia as compared with basal value. Neither the RNA nor the DNA concentrations were significantly changed in septicemia. The RNA/DNA ratios remained unchanged (Table II).

Haemanalyses. A reduction in arterial leucocyte concentration was seen in early and late sepsis. It was mainly due to pronounced decrease in polynuclear cells (Table III). The platelet concentrations decreased in the septic and the control cats. pH decreased late in the septic cats, while pO_2 remained unchanged and pCO_2 declined in both groups (Table IV).

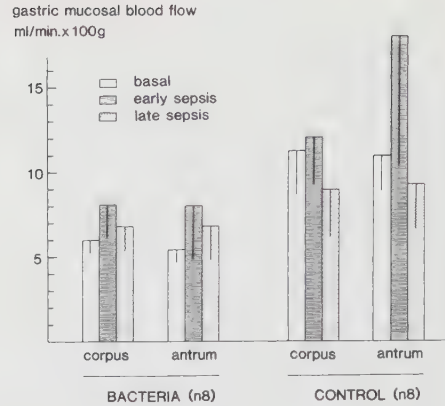


Fig. 3. Gastric mucosal blood flow in the corpus and antrum area in cats with induced bacteraemia and in controls. The differences from basal values were not statistically significant, nor was the difference between basal value in bacteraemic cats and controls. Mean values $\pm$ SE.

DISCUSSION

In this study the animals were challenged with the combination of significant surgical trauma and bacteraemia. The surgical trauma alone was sufficient to cause hypotension in four control cats. In earlier studies, however, such combined surgical and septic challenge, or that challenge in combination with intragastric bile and/or pentagastrin stimulation, did not induce gastric mucosal lesions (1, 2). By contrast, topical administration of 80 mM HCl and bile together with intravenous bacterial infusion regularly induced gastric mucosal damage (1). In accordance with earlier reports, therefore, acid and bile together with a stress factor were necessary to induce gastric lesions (5, 18, 26).

In the present experiments, central haemodynamics, gastric and gastric mucosal blood flow, collagen concentration of the gastric wall and

Table I. Histologic grading of gastric mucosal lesions

Group	Index of gastric mucosal damage	
	0-5	6-12
Bacteraemia (n=8)	0	8
Controls (n=11)	10	1

Table II. Collagen ($\mu\text{g}/\text{mg}$ dry tissue) in the gastric wall, and RNA, DNA ($\mu\text{g}/100$ mg wet tissue) and RNA/DNA ratio in gastric mucosa before and after bacteraemia (means $\pm$ SE)

Group	Collagen	RNA	DNA	RNA/DNA
Bacteraemia ($n=8$)				
Before bacterial infusion	59.6 $\pm$ 7.0	35 $\pm$ 14	210 $\pm$ 77	0.21 $\pm$ 0.05
After bacterial infusion	53.9 $\pm$ 3.2	29 $\pm$ 10	184 $\pm$ 66	0.26 $\pm$ 0.06
Controls ($n=11$)				
Before saline infusion	48.4 $\pm$ 4.8	32 $\pm$ 7	284 $\pm$ 55	0.13 $\pm$ 0.02
After saline infusion	47.2 $\pm$ 4.7	32 $\pm$ 6	233 $\pm$ 39	0.15 $\pm$ 0.02

Table III. Leukocytes and platelets ($\times 10^6/\text{l}$) before, and early and late after infusion of bacteria (8 cats) or saline (controls, 11 cats), means $\pm$ SE

	Basal value	Early sepsis	Late sepsis
Polynuclear leucocytes			
Bacteraemic cats	6.6 $\pm$ 1.0	2.1 $\pm$ 0.5*	0.9 $\pm$ 0.2*
Controls	8.6 $\pm$ 2.3	7.4 $\pm$ 1.0	11.4 $\pm$ 1.8
Mononuclear leucocytes			
Bacteraemic cats	3.6 $\pm$ 0.5	1.4 $\pm$ 0.3*	2.1 $\pm$ 0.7
Control cats	2.4 $\pm$ 0.6	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1*
Platelets			
Bacteraemic cats	157.2 $\pm$ 32.6	78.2 $\pm$ 16.5*	119 $\pm$ 21.0*
Controls	130.0 $\pm$ 11.8	75.9 $\pm$ 11.4*	115.2 $\pm$ 19.8*

* Significant difference ($p<0.05$) from basal value.

RNA/DNA were measured in bacteraemic and control cats given bile and HCl intragastrically. The central haemodynamic and the hematologic responses, i.e. profound hypotension, reduced cardiac output, increased pulmonary arterial blood pressure and diminished counts of arterial leucocytes and platelets, were similar to the pattern of reactions described by previous authors (9) who used a closely similar model of septic shock.

Reduced mucosal blood flow has been discussed as a pathogenetic factor in acute gastric mucosal damage. Mucosal ischaemia was stated to be a prerequisite for development of gastric mucosal lesions in haemorrhagic (3, 14, 18, 28) and endotoxin (8) shock. Other workers, however, demonstrated gastric mucosal lesions in septicemic dogs with gastric blood flow remaining at, or exceeding the pre-septic level (23, 25). In piglets, intravenous in-

Table IV. $p\text{O}_2$, $p\text{CO}_2$ (kp) and pH before, early and late after infusion of bacteria (8 cats) or saline (controls, 11 cats), means $\pm$ SE

	Basal value	Early sepsis	Late sepsis
$p\text{O}_2$			
Bacteraemic cats	14.2 $\pm$ 0.5	15.8 $\pm$ 1.1	15.0 $\pm$ 0.5
Controls	14.7 $\pm$ 0.6	15.5 $\pm$ 0.7	16.0 $\pm$ 0.7
$p\text{CO}_2$			
Bacteraemic cats	3.8 $\pm$ 0.1	3.6 $\pm$ 0.2	2.9 $\pm$ 0.2*
Controls	4.2 $\pm$ 0.4	3.5 $\pm$ 0.1*	3.2 $\pm$ 0.1*
pH			
Bacteraemic cats	7.41 $\pm$ 0.03	7.39 $\pm$ 0.04	7.25 $\pm$ 0.06*
Controls	7.42 $\pm$ 0.03	7.45 $\pm$ 0.02	7.32 $\pm$ 0.04

* Significant difference ($p<0.05$) from basal value.

jection of live *E. coli* was reported to induce increase of the gastric mucosal blood flow and gastric mucosal damage (10). Also in the present experiments, coeliac artery blood flow remained in the preseptic range, and it was even less in the control group than in the bacteraemic cats. Furthermore, gastric mucosal blood flow in early and late sepsis, evaluated with the microsphere technique, remained in the presepsis range and there was no significant difference in this respect between the bacteraemic and the control cats. A weak correlation ($r=0.58$; $p<0.05$) nevertheless was found between the lesion index and the gastric mucosal blood flow in late sepsis. The degree of hypotension was also found to be associated with development of gastric mucosal lesions. Thus there was significant difference in lesion index between normotensive and hypotensive cats ($p<0.02$). Arterial acidosis likewise was associated with mucosal damage ($r=0.69$; $p<0.01$), probably reflecting the importance of decreased mucosal tissue pH for the development of gastric mucosal lesions, as earlier pointed out (18, 28).

The mucosal damage found in our study was confirmed to the parietal cell-bearing area of the stomach. As a rule the greater curvature was affected, but not the lesser. The areas selected for histologic study (greater curvature, anterior and posterior fundic wall) thus represented the sites most commonly damaged, but with different frequencies. The determinations of mucosal blood flow were made in the same areas.

The data did not permit comparisons between blood flow in the major and the minor curvature. The antral mucosa was always normal. Antral, or antral mucosal, blood flow was not different from that of the corpus. The antral mucosa was previously demonstrated to be more resistant to acute lesion formation, possibly because of a smaller energy requirement (22).

Alterations in nucleic acid metabolism have been proposed as prerequisites for the formation of acute gastric lesions (15). Following restraint and stress, loss of RNA and decreased DNA-synthesis were reported to be coincident with gastric mucosal erosions (15, 19, 30). Our experiments failed to demonstrate changes in mucosal nucleic acid concentration or in RNA/DNA ratio. This would seem to contradict nucleic acid changes as a *primary* cause of gastric mucosal damage in response to septic stress. A pertinent point is that the studied speci-

mens were taken from macroscopically normal mucosa, so as to avoid interference from necrotic tissue.

Breakdown of collagen in the gastric wall may also be a factor in the pathogenesis of acute damage to the gastric mucosa. Changes in collagen could conceivably follow a septic challenge. Such changes were recently described in connection with healing of anastomoses and incisional wounds in the gastrointestinal tract (11, 16, 17). We therefore wished to observe if the development of acute mucosal lesions was associated with increased collagenolysis. In the present experimental setting, however, no changes in collagen concentration were found.

Acute mucosal ulcerations of the stomach are generally considered as a single entity, and different forms of stress—such as septic and haemorrhagic shock—are thought to act via the same pathophysiologic mechanisms. This is not necessarily true. The gastric haemodynamic reactions in haemorrhagic shock are characterized by a reduction in the mucosal blood flow (3, 5, 14). In septic shock, as illustrated also in our study, the gastric mucosal blood flow remains unchanged or increased (10). Lesions of the gastric mucosa appear in both states of shock. It would thus seem that sepsis and haemorrhage can induce different changes in tissue microcirculation, tissue energy, oxygen demand and supply, possibly accounting for acute gastric mucosal damage of differing pathogenesis in septic and in haemorrhagic shock.

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GASTRIC MUCOSAL DAMAGE IN SEPSIS - EFFECTS OF PRETREATMENT WITH
A SYNTHETIC PROSTAGLANDIN E₁ ANALOGUE

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ABSTRACT

An experimental model was used which includes intragastric instillation of 80 mM HCl and 0.6 ml bile/kg followed by i.v. infusion of live *E.coli* in cats for up to three hours. This procedure regularly induces gastric mucosal ulcerations. Mucosal blood flow was measured by microspheres before, early and late in sepsis. Total gastric blood flow was recorded electromagnetically. Mucosal regeneration capacity as reflected by the RNA/DNA ratio was measured. Misoprostol (a PGE₁ analogue) was infused i.v. (5 µg/kg x h) or given locally in the stomach before bactriemia. Misoprostol did not influence the hemodynamic response to bacteria. The gastric mucosal damage was assessed either as an index representative for the entire corpus-fundus region or as the number of areas with intact surface epithelium within the series. Misoprostol i.v. protected the mucosa from ulceration compared to untreated septic controls while misoprostol i.g. significantly reduced the number of damaged areas only. Topical misoprostol increased total gastric and mucosal blood flows early in sepsis compared to i.v. or no pretreatment while no difference was seen during late sepsis. The protective effect of misoprostol was thus not dependent on increased gastric mucosal blood flow. Nor was it mediated through effects on mucosal nucleic acid concentrations or ratio.

INTRODUCTION

Prostaglandins in doses that have no effect on acid production protect the gastric mucosa from damage due to stress of various kinds. This "cytoprotective" effect (1) has been well documented in several studies during the last two decades (2,3). The mechanism underlying the cytoprotective effect of prostaglandins remains, however, unclear.

Acute gastric mucosal damage (stress ulcer) is a potentially dangerous complication in septicemia (4,5). Cytoprotective agents might be of importance in the prevention of the development of these lesions. Misoprostol, a novel PGE_1 analogue (G.D. Searle Co Ltd, USA) has been found to have gastric antisecretory and cytoprotective properties (6,7).

The aim of this series of experiments was to investigate if intragastrically or intravenously administered misoprostol exhibited protective effects on the gastric mucosa in a standardized feline septic shock model and to relate this cytoprotective effect, if any, to effects on regional blood flow, mucosal regeneration capacity, and central hemodynamics.

MATERIAL AND METHODS

The experiments were performed on 20 cats, weighing 1.9 - 4.7 kg. They were deprived of food for 24 hours with free access to water. Anesthesia was induced by approximately 50 mg pentobarbital i.v. (PENTOTHAL^R) and continued with chloralose (50 mg/kg b.w.).

Operative procedures, determination of gastric mucosal blood flow, histologic examination and biochemical analysis have been previously described in detail (8). Briefly, the animals were tracheotomized and ventilated artificially. A thoracotomy was performed to allow injection of radioactively labelled microspheres in the left atrium. A SwanGanz catheter (5F, Edwards Laboratories, Santa Ana, California, USA) was used for monitoring cardiac output (thermodilution; Edwards computer 1520) and pulmonary artery blood pressure (PAP). Systemic arterial blood pres-

sure was recorded and blood samples were drawn from catheters in the femoral arteries. Infusions were given in the brachial veins. After laparotomy and splenectomy the hepatic artery was ligated proximal to the gastroduodenal artery allowing collateral arterial circulation to the liver from the superior mesenteric artery. An electromagnetic flow probe (Cliniflow model 601D, Carolina Medical Electronics Inc, King, North Carolina, USA) was put on the coeliac artery for continuous measurements of approximately total gastric blood flow. An intragastric tube was introduced through a duodenotomy and connected to a reservoir keeping the intragastric pressure constant at 2 cm of water. An i.v. infusion of glucose containing bicarbonate (10 mmol/100 ml 5.5% glucose; 0.1-9.2 ml/min) was continued throughout the experiment.

Determination of gastric mucosal blood flow. Approximately 900,000 microspheres labelled with ^{141}Ce , ^{103}Ru or ^{46}Sc in 10% dextran containing 0.05% of Tween 80 were injected in random order before, 15 min and 150 min after the start of bacterial infusion. However, if the arterial blood pressure decreased to a level of 50 mmHg the last injection was made at that time (range 70-150 min). The time for injection is therefore referred to below as "early" and "late" sepsis. A reference blood flow sample was withdrawn from the thoracic aorta at a speed of 3.4 ml/min (= reference flow) starting 10 s before microsphere injection and continued for 70 s (for further details see 8). At the end of the experiments parts of the fundus, major curvature and antrum were excised, the mucosa dissected free and dissolved in concentrated sulphuric acid. Radioactivity was measured in a gamma scintillation counter (Ultragamma^R). Blood flow was given by the formula: Tissue blood flow (ml/min x 100 g tissue) =

$$\frac{\text{tissue radioactivity} \times \text{reference flow}}{\text{reference radioactivity}}$$

Histological examination

After the experiments the stomach was rapidly removed and tissue secured for gamma counting. The remainder was mounted on cork, fixed in 4% buffered formaline, prepared for paraffin embedment, cut at 3-4 μm , mounted and stained with hematoxylin-eosine. The glasses were coded for histological examination. The gastric mucosal lesions were graded as described previously (8).

Grade 0 = normal mucosa,
grade 1 = oedema beneath the superficial epithelium,
grade 2 = disappearance of the surface epithelial cells,
grade 3 = damage of the upper half of the glands,
grade 4 = disappearance of the glands.

A gastric mucosal damage index was calculated as the sum of the maximal damage in three predetermined corpus areas (anterior wall, posterior wall and major curvature). Thus, a gastric index varying from 0 to 12 was obtained in each animal. In addition, the gastric mucosal damage was assessed by counting the number of areas with intact epithelium (grade 0 - 1) in the different series.

RNA and DNA analysis. A 5 x 5 mm biopsy was obtained from the mucosa of the anterior gastric body before sepsis induction and analyzed for RNA and DNA concentration. At the end of experiments two additional biopsies were also obtained from the posterior wall and major curvature of the corpus. The mean of these two values gave the mucosal RNA and DNA concentration after sepsis. RNA was determined according to the method described by Ceriotti (9) and DNA according to the method of Burton (10). Standards were obtained from Sigma Chemical Company, St Louis, USA.

Bacterial preparation

An *Escherichia coli* strain (E.coli O6K 13H1) from WHO Collaborative Centre for Reference and Research on *Escherichia* (State Serum Institute, Copenhagen, Denmark) was used. The bacteria were cultivated in nutrient broth. Just prior to the infusion, the bacteria were washed in saline, resuspended in 0.9% NaCl, and infused at a concentration of 10^9 live cells/ml. All cats received 1 ml/kg x min for 2 min and then 1 ml/kg x hour for up to three hours.

Experimental procedures

After preparation the cats were allowed to stabilize for 30 min. One minute prior to the i.v. infusion of live E.coli, 0.6 ml bile/kg (obtained from the cat gall bladder) and HCl at a concentration of 80 m.molar (volume adjusted to keep intragastric pressure at 2 cm of water, see above) were instilled into the

stomach in all cats. In one group of animals, no pretreatment was given ($n=8$, controls). Six other cats received a continuous i.v. infusion of $5 \mu\text{g/kg} \times \text{hour}$ of misoprostol dissolved in 2.5 ml phosphate buffer (pH 7.4) starting 10 min before the induction of sepsis and continued throughout the experiment. Another 6 animals were given $10 \mu\text{g/kg}$ of misoprostol in saline administered intragastrically (i.g.) 10 min before the sepsis induction. Arterial blood was sampled for determination of platelets and white blood cells, pH, pO_2 and pCO_2 before drugs, at early, and at late sepsis.

Statistical methods

Data are expressed as mean $\pm$ SE if based on five or more observations; otherwise the mean value alone is given. Ordinal scale data are given as median and interquartile range (i.r.). When testing statistical significance the nonparametric methods (the Wilcoxon's matched-pair signed-rank test and the Fischer exact probability test) described by Siegel (11) and Wilcoxon's rank test were used. A p-level < 0.05 was considered as significant.

RESULTS

Hemodynamics. In the preseptic period arterial blood pressure was 126 ± 9 mmHg in controls; 120 ± 14 and 116 ± 9 for i.v. and intragastric (i.g.) misoprostol groups, respectively. Misoprostol administered either i.v. or i.g. had no effects on "resting" blood pressure. At the end of the experimental period 6 of 8 control cats and 4 of 6 in both misoprostol groups were hypotensive or not alive (fig 1). Pulmonary artery blood pressure increased from 14 to 34 mmHg 3 minutes after bacteria, normalizing at 15 min, and was 21 mmHg at late sepsis in control (bacteria only); similar pressure levels were obtained in the i.v. and i.g. misoprostol series, respectively.

Cardiac output responded similarly in the three groups. A rapid increase was seen at 1 min after the start of bacterial infusion followed by a progressive fall to about 60% of basal value at the end (fig 1).

Coeliac artery blood flow increased by more than 100% in animals

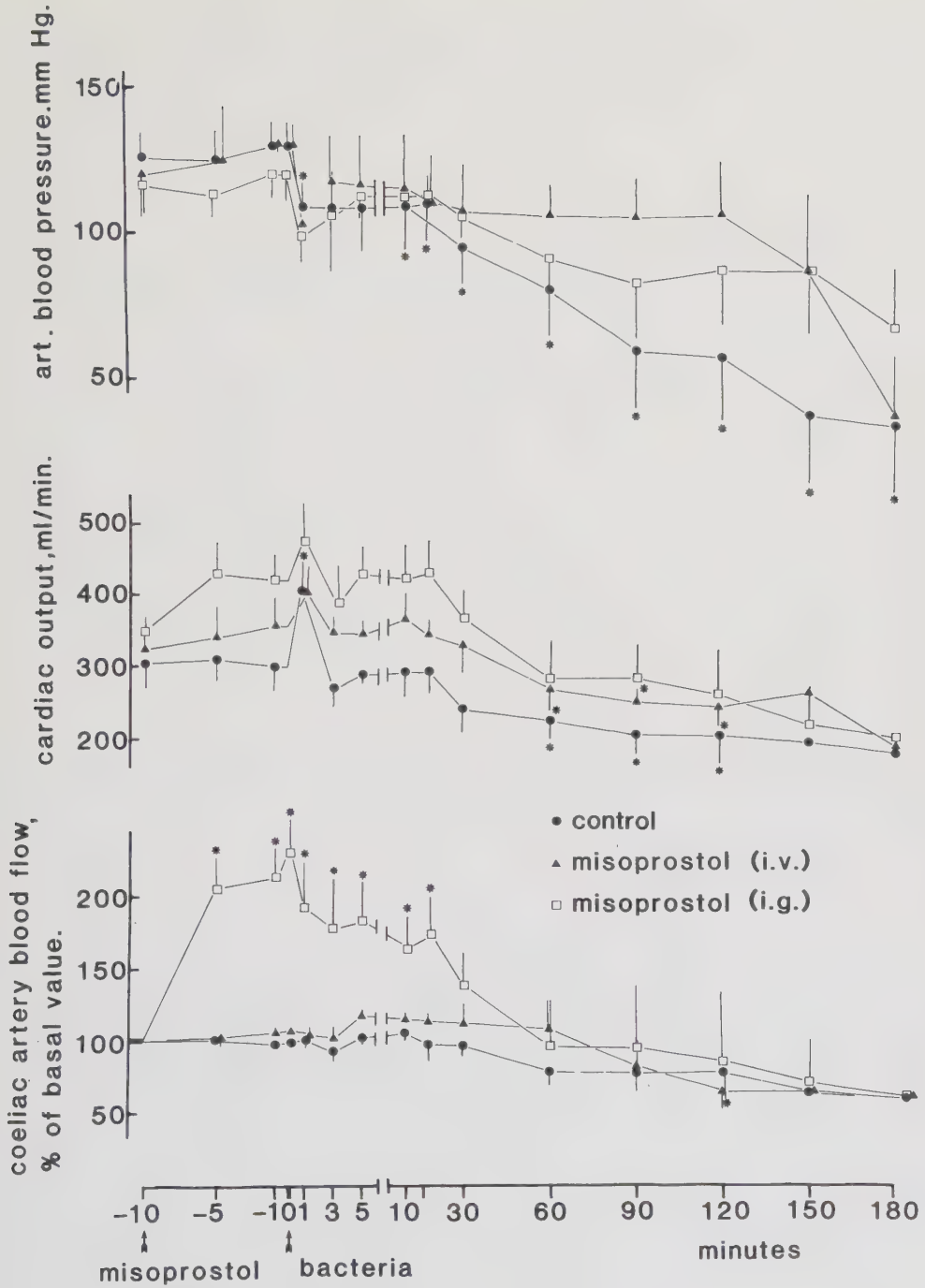


Fig 1: The effects of misoprostol pretreatment on arterial blood pressure, cardiac output and coeliac artery blood flow in septic cats. * denotes significant difference compared to basal value ($p < 0.05$).

with local administration of misoprostol into the stomach. This vasodilatory effect lasted for about 1 hour from the instillation of the drug (fig 1). In animals without pretreatment or given i.v. misoprostol coeliac artery blood flow was unchanged.

Gastric lesions. All cats without treatment (control) had disrupted superficial epithelium giving lesion index 6 or more (median 8 i.r. 7-8). In i.v. misoprostol series the mucosal index was 5 or less in 4 of 6 cats (median 4 i.r. 3-7; $p < 0.05$ compared to control, Fisher exact probability test). In the i.g. misoprostol group 3 animals had an index of 5 or less while 3 had more pronounced lesions (median 5 i.r. 3-6) and the gastric damage index in this group was not statistically different from control. Table I details the grading of the gastric mucosal lesions in the three different areas that were examined. In both i.g. and i.v. misoprostol pretreated groups significantly more such areas were found with intact superficial epithelium (grade (0-1); $p < 0.002$ for both i.v. and i.g. misoprostol compared to controls).

TABLE I

Grade lesion in anterior wall, posterior wall and major curvature. Grade 0 and 1 means intact superficial epithelium. ● = control, o = i.v. misoprostol and x = intragastric misoprostol.

		Grade				
		0	1	2	3	4
anterior wall			●	●●●●●	●●	
	o		ooo xxxxx		oo x	
posterior wall			●	●●●	●●●	●
	oo		oo xx	xx	oo x	x
major curvature				●●●	●●	●●●
	o x		ooo xxx		o x	o x

Gastric mucosal blood flow was unchanged in controls and i.v. misoprostol group as measured "early" or "late" after sepsis induction (fig 2). In contrast, local administration of misoprostol increased mucosal blood flow from 8.4 ± 1.2 to 25.1 ± 9.9 ml/min $\times$ 100 mg tissue in corpus/fundus and from 6.7 ± 0.8 to 47.8 ± 9.2 in antrum in early sepsis (fig 2).

The nucleic acid content or the RNA/DNA ratio were neither influenced by sepsis nor by misoprostol during the period of three hours of the septic state in any of the three groups (Table II). One of the control animals was excluded because of technical failure.

gastric mucosal blood flow
ml/min $\times$ 100mg

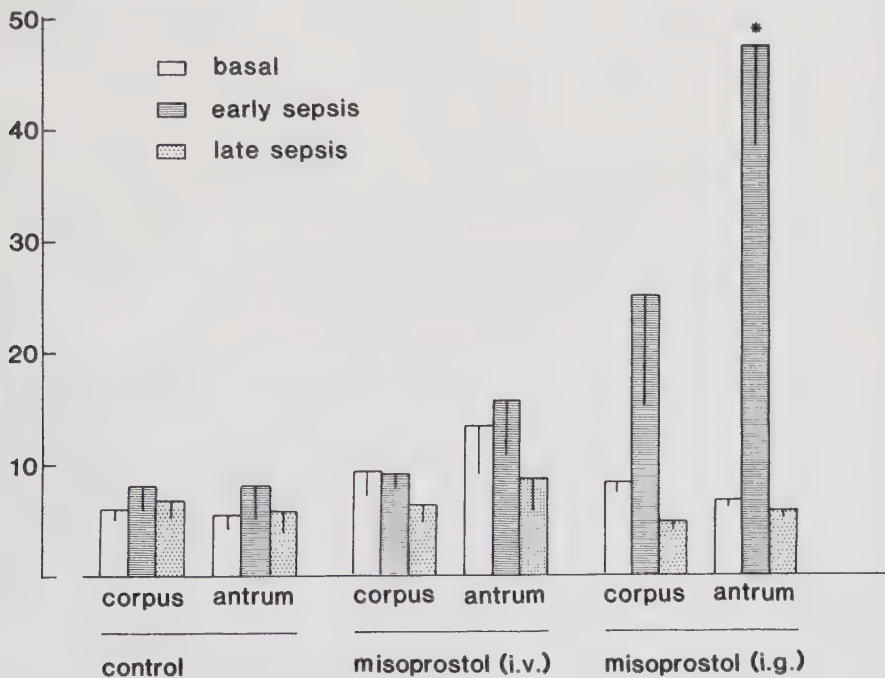


Fig 2: Gastric mucosal blood flow in misoprostol pretreated animals and controls. For early and late sepsis see text. Early sepsis flow in intragastric misoprostol group was increased both in antrum ($p < 0.01$) and corpus ($p < 0.05$) compared to control. * denotes significant difference compared to basal value ($p < 0.05$).

TABLE II

RNA, DNA ($\mu\text{g}/100 \text{ mg}$ tissue wet weight) and RNA/DNA ratio in gastric mucosa before and after bacteriemia (mean values $\pm$ SE).

	RNA	DNA	RNA/DNA
Control			
before bacteria	37 $\pm$ 16	235 $\pm$ 84	0.16 $\pm$ 0.02
after bacteria	31 $\pm$ 11	207 $\pm$ 71	0.21 $\pm$ 0.04
Misoprostol i.v.			
before bacteria	47 $\pm$ 14	441 $\pm$ 90	0.10 $\pm$ 0.02
after bacteria	32 $\pm$ 7	320 $\pm$ 34	0.10 $\pm$ 0.02
Misoprostol i.g.			
before bacteria	40 $\pm$ 12	367 $\pm$ 51	0.11 $\pm$ 0.03
after bacteria	25 $\pm$ 4	229 $\pm$ 26	0.12 $\pm$ 0.03

TABLE III

Arterial blood pH at early and late sepsis in control (n=8), misoprostol i.v. (n=6) and misoprostol i.g. (n=6) animals before and after E.coli infusion (mean value $\pm$ SE). * denotes significant difference compared to basal value.

	pH		
	Basal	Early	Late
Control	7.41 $\pm$ 0.03	7.39 $\pm$ 0.04	7.25 $\pm$ 0.06*
Misoprostol i.v.	7.41 $\pm$ 0.01	7.40 $\pm$ 0.04	7.30 $\pm$ 0.06*
Misoprostol i.g.	7.43 $\pm$ 0.01	7.40 $\pm$ 0.03	7.20 $\pm$ 0.07*

Blood tests. All three groups were acidotic at "late" sepsis (Table III). pO_2 was 14.2 ± 0.5 , 16.6 ± 1.0 and 14.7 ± 1.5 kP in the three groups, respectively, before bacteria and did not change during the experiments. pCO_2 decreased from 3.8 ± 0.1 to 2.9 ± 0.2 kP at "late" sepsis in cats without pretreatment and from 3.9 ± 0.2 to 2.7 ± 0.4 kP in i.g. misoprostol group. Corresponding values for i.v. misoprostol were 3.7 ± 0.3 and 3.4 ± 0.2 . Misoprostol did not affect the rapid reduction of circulating arterial white blood cells or platelets (Table IV).

TABLE IV

Leucocytes (polymorphonuclear and mononuclear; $\times 10^6/l$) and platelets ($\times 10^6/l$) in control (n=8), misoprostol i.v. (n=6) and misoprostol i.g. (n=6) before, early and late (see text) after bacterial infusion (mean values $\pm$ SE). * denotes statistically significant difference ($p < 0.05$) compared to basal value.

	Basal value	Early sepsis	Late sepsis
Polynuclear leucocytes			
Control	6.6 ± 1.0	$2.1 \pm 0.5^*$	$0.9 \pm 0.2^*$
Misoprostol i.v.	9.2 ± 1.8	$3.7 \pm 1.8^*$	$1.3 \pm 0.3^*$
Misoprostol i.g.	7.3 ± 1.0	$2.5 \pm 0.6^*$	$1.0 \pm 0.2^*$
Mononuclear leucocytes			
Control	3.6 ± 0.5	$1.4 \pm 0.3^*$	2.1 ± 0.7
Misoprostol i.v.	1.1 ± 0.3	$0.5 \pm 0.2^*$	0.8 ± 0.2
Misoprostol i.g.	1.6 ± 0.3	$0.6 \pm 0.2^*$	$0.7 \pm 0.2^*$
Platelets			
Control	157.2 ± 32.6	$78.2 \pm 16.5^*$	$119.1 \pm 21.0^*$
Misoprostol i.v.	102.9 ± 19.8	$29.9 \pm 6.8^*$	72.8 ± 13.0
Misoprostol i.g.	125.1 ± 25.9	$59.5 \pm 16.3^*$	84.0 ± 13.2

DISCUSSION

In this experimental model live *E. coli* septicemia induced pronounced gastric mucosal lesions. Misoprostol administered i.v. protected the mucosa significantly while the intragastric administration of misoprostol prior to sepsis did not result in significant reduction of total gastric lesion index. However, if comparing number of areas in the stomach with intact superficial epithelium to those without intact epithelium, a significant effect was seen also with the locally administered prostaglandin. The doses given i.v. or i.g. were in experiments on conscious dogs demonstrated to be equipotent in so far as the inhibition of gastric secretion is concerned (11). However, it is very unlikely that the acid secretion inhibitory property of misoprostol is of any importance in the present experimental model in which exogenous acid is instilled into the stomach. Since we do not know whether the doses given are equipotent as concerns "cytoprotective" effects or not, a strict comparison between i.v. and i.g. misoprostol cannot be made. Intragastric misoprostol increased mucosal and total gastric blood flow at early sepsis in contrast to i.v. misoprostol, which had no such effect. Thus, in the present shock model, topical misoprostol provided limited mucosal protection together with increased gastric and gastric mucosal blood flow. On the other hand, i.v. misoprostol had no effect on flow and despite this, the mucosa was significantly protected from ulcerations. This indicates that other mechanisms besides increased mucosal blood flow are important in the protection against gastric mucosal lesions in septic shock. The background to the difference in effect on gastric blood flow between i.v. and i.g. misoprostol could possibly be differences in local concentration, but this has not been further investigated.

As discussed previously (8) gastric ulcerations develop in this septic shock model without signs of gastric or gastric mucosal ischemia. This is contrary to the currently accepted pathogenesis for stress ulcers as outlined e.g. by Silen et al (13). The view that mucosal ischemia is not an important factor in the pathogenesis of acute gastric ulcerations in sepsis is, however, in agreement with other recent data. Genter et al (14), studying the effects of *E. coli* injected i.v. in piglets, found an increased mucosal blood flow as measured by microspheres. This response

failed to appear in animals pretreated with indomethacin, proposing prostaglandins as a mediator of the vascular reactions in the septic gastric mucosa (15). Rees et al (16) found no difference in gastric venous outflow between ulcerated and nonulcerated septicemic dogs, also indicating that gastric blood flow is of less importance. However, these authors proposed "functional shunting" in the mucosa, resulting in apical gland ischemia, causing the mucosal damage. The present technique does not allow studies of blood flow distribution within the mucosa.

The cytoprotective effect of prostaglandins on the gastric mucosa exposed to external injurious agents like indomethacin, acetylsalicylic acid, boiling water etc, is well documented (1,3). Misoprostol gave mucosal protection in aspirin induced gastric damage in dogs (6,17) and in stress induced gastric ulcer formation in rats (18). The potentially protective effects of prostaglandins in more "physiological" forms of stress, such as sepsis or hemorrhagic shock, have not attracted similar attention.

Odonkor et al (19) found cytoprotective properties of parenterally given 16,16-dimethyl PGE_2 on mucosal damage in peritonitis induced septicemia in conscious dogs. Using a canine hemorrhagic shock model, including topical aspirin, lesion formation was significantly reduced by misoprostol but not by 16,16-dimethyl PGE_2 . Misoprostol decreased the shock-induced transmucosal potential difference and increased gastric venous outflow. It was concluded that protective effects of misoprostol were mediated via increased blood flow (7).

Regenerating capacity of gastric mucosal cells has been proposed to be of importance in the development of stress ulcerations in rats and mice (21,22). 16,16-dm PGE_2 has been shown to prevent the reduction in RNA and DNA in rat gastric mucosa exposed to 100% ethanol (22). Changes in RNA/DNA ratio reflect increased mucosal regeneration activity (23). However, in the present series of experiments no changes were observed in nucleic acid concentration of the gastric mucosa or RNA/DNA ratio in response to the sepsis. Nor did misoprostol, regardless of the route of administration, affect RNA or DNA concentration of the gastric mucosa (Table II).

Misoprostol delayed systemic hypotension but had otherwise no significant effect on the central hemodynamic or hematological reaction to bacterial infusion. The results of studies using cyclooxygenase (prostaglandin synthetase) inhibitors as well as of those using exogenous prostaglandin supply are contradictory (24). Cyclooxygenase inhibitors have been reported to prevent the pulmonary hypertension (25) and the pulmonary platelet trapping (26) in septic states. The lack of effect on pulmonary circulation by misoprostol supports the view that other arachidonic acid metabolites, such as thromboxan A_2 and prostacyclin (PGI_2), are of more significance for the pulmonary response to sepsis.

In conclusion, gastric lesions in a feline septic shock model were significantly diminished, but not prevented, by misoprostol. The protective effect was not mediated through increased blood flow or effects on nucleic acids of the mucosa.

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ROLE OF OXYGEN FREE RADICALS IN THE DEVELOPMENT OF GASTRO-
INTESTINAL MUCOSAL DAMAGE IN E.COLI SEPSIS

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ABSTRACT

Live *E.coli* were infused in anesthetized cats. Arterial and pulmonary artery blood pressures, cardiac output, and gastric blood flow were monitored. Gastric, small intestinal and colonic total wall blood flow and gastric and small intestinal mucosal blood flow were measured by microspheres. Before the experiments 0.6 ml bile and 80 mM HCl were instilled in the stomach. The microscopic mucosal damage was graded 0-4 in the gastric- and 0-5 in the small and the large intestinal mucosa. One group of cats (n=8) received 5 mg yeast CuZn superoxide dismutase (SOD) as a bolus before bacteria followed by infusion 50 mg/3 h. Four of these cats were also given catalase in the same dose. A control group (n=8) had no treatment. After up to three hours septicemia gastric ulceration (grade 2-4) was found in all controls but only in 50% of treated cats ($p<0.1$). About 50% and 25% of the cats developed significant, small intestinal and colonic mucosal damage, respectively. There was no difference between controls and treated cats. There was no effect of SOD or SOD/catalase on the late systemic hypotension, the decrease in cardiac output, transient increase in pulmonary pressure. Total gastric blood flow did not change in either group. At late sepsis gastric mucosal flow was decreased in the treated group but not in controls. Small intestinal mucosal flow decreased in both series. It is concluded that free oxygen radicals may be of partial importance in the development of sepsis induced gastric, but not intestinal, mucosal damage.

INTRODUCTION

Most of the oxygen consumed in the body is directly reduced to water in four steps in the mitochondrial cytochrome chain. However, a few percent is reduced through other mechanisms and for quantum mechanistic reasons these reductions occur one step at a time leading to the formation of a series of toxic oxygen reduction products. In sequence the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^\cdot$) are formed. The toxic oxygen reduction products have the potential to damage all biological components in the body (1,2).

The toxic oxygen reduction products have been implicated in the development of small intestinal mucosal lesions in regional ischemia and reperfusion experiments (3,4). In live *E. coli* sepsis tissue damage occurs in the gastro-intestinal mucosa as previously shown in cats (5,6). The aim of the present study was to examine whether toxic oxygen reduction products contribute to the formation of these lesions. The occurrence of lesions in animals treated with parenteral superoxide dismutase and catalase as compared to controls was studied in a standardized feline septic shock model. Superoxide dismutase disproportionates the superoxide anion radical to hydrogen peroxide and oxygen ($2O_2^- + 2H^+ > O_2 + H_2O_2$), and catalase disproportionates hydrogen peroxide to water and oxygen ($2H_2O_2 > O_2 + 2H_2O$). The combination of these enzymes therefore has the potential to lead to significant reduction of damage if toxic oxygen reduction products are involved.

MATERIAL AND METHODS

The experiments were performed on 16 cats, weighing 2.4 - 4.6 kg. They were deprived of food for 24 hours but had free access to water. Anesthesia was induced by approximately 50 mg pentobarbital i.v. (Pentothal^R) and continued with chloralose (50 mg/kg).

Operative procedures, determination of regional blood flow and histologic examination of the gastric mucosa have previously been described in detail (5). Briefly, the animals were tracheo-

stomized and ventilated artificially. A thoracotomy was performed to allow injection of radioactively labelled microspheres in the left atrium of the heart. A Swan-Ganz catheter (5F, Edwards Laboratories, Santa Ana, California, USA) was introduced via the right femoral vein and used for monitoring cardiac output (thermodilution; Edwards computer 1520) and pulmonary artery blood pressure (PAP). Catheters were introduced in the femoral arteries for monitoring arterial blood pressure and blood sampling. After laparotomy and splenectomy the hepatic artery was ligated proximal to the gastroduodenal artery; allowing collateral circulation to the liver from the superior mesenteric artery. An electromagnetic flow-probe (Cliniflow, model 601D, Carolina Medical Electronics Inc, King, North Carolina, USA) was placed on the coeliac artery for continuous monitoring of approximately total gastric blood flow. Through a duodenotomy a tube was introduced intragastrically and, after ligation of the pyloric ring, connected to a reservoir to keep the intragastric pressure constant at 2 cm of water. Infusions were given in the brachial veins. An i.v. infusion of glucose containing bicarbonate (10 mmol/100 ml 5.5% glucose; 0.1-0.2 ml/min) was continued throughout the experiment. Arterial blood was sampled for determination of white blood cells and pH before, at early and at late sepsis.

Determination of tissue blood flow. Approximately 900,000 microspheres labelled with ^{141}Ce , ^{103}Ru and ^{46}Sc in 10% dextran containing 0.05% of Tween 80 were injected in random order before, 15 and 150 min after the start of bacterial infusion. However, if the arterial blood pressure decreased to a level of 50 mm Hg, the last injection was made at that time (range 70-150 min). The time for injection is therefore referred to below as "early" and "late" sepsis. A reference blood flow sample was withdrawn from the thoracic aorta at the speed of 3.4 ml/min starting 10 sec before the microsphere injection and continued for 70 sec. At the end of the experiments two parts of the gastric corpus mucosa, one part of the antral mucosa and two parts of the midportion of the small bowel mucosa were dissected free. From the midportion of the small and large bowel two total wall pieces were obtained. Each specimen, weighing about 1 g, was dissolved in 3 ml concentrated sulphuric acid. Radioactivity was measured in a gamma scintillation counter (Ultragamma^R). Blood flow was given by the form-

$$\text{ula: Tissue blood flow (ml/min} \times 100 \text{ g tissue)} = \frac{\text{tissue radioactivity} \times \text{reference flow}}{\text{reference radioactivity}}$$

In the case where two samples were obtained, flow was calculated as the mean of these values.

Histologic examination

After the experiments the stomach and the small and large intestine were rapidly removed and tissue secured for gammacounting. The remainder of the stomach, a 4 cm long piece of the small intestine 20 cm from lig. of Treitz and a 3 cm specimen from the midportion of the large intestine were mounted on cork, fixed in 4% buffered formaline, prepared for paraffin embedment, cut at 3-4 μm , mounted and stained with hematoxylin-eosin. The glasses were coded for histologic examination.

The gastric mucosal lesions were graded 0 - 4 as previously described (5):

Grade 0 = normal mucosa

Grade 1 = edema beneath the superficial epithelium

Grade 2 = disappearance of the surface epithelial cells

Grade 3 = damage of the upper half of the glands

Grade 4 = disappearance of the glands

A gastric mucosal lesion index was calculated as the sum of the maximal damage in three predetermined corpus areas (anterior wall, posterior wall and major curvature). Thus, a gastric index varying from 0 - 12 was obtained in each animal. In addition, the gastric mucosal damage was assessed by counting the number of areas with intact superficial epithelium (grade 0 - 1) in the different series.

The small intestinal mucosal lesions were graded as described earlier (6-8):

Grade 0 = normal mucosa

Grade 1 = development of a subepithelial space at the tip of the villus

Grade 2 = epithelial lifting at the tip of the villus

Grade 3 = massive epithelial lifting down the sides of the villus

Grade 4 = denuded villus

Grade 5 = disintegration of the mucosal lamina propria, hemorrhage and ulceration

The large intestinal mucosal lesions were graded as follows:

Grade 0 = normal mucosa

Grade 1 = subepithelial space at the top of lamina propria

Grade 2 = epithelial lifting from lamina propria

Grade 3 = massive epithelial lifting from lamina propria down the crypts of Lieberkühn

Grade 4 = destruction of the epithelium in the crypt of Lieberkühn with denuded lamina propria

Grade 5 = hemorrhage, ulceration and disintegration of the lamina propria

Grade 2 - 5 was considered as significant damage for both small and large intestine.

Bacterial preparation

An *Escherichia coli* strain (E.coli 06K 13H1)(WHO designation SU 4344/41) from the WHO collaborative center for Reference and Research on *Escherichia* (State Serum Institute, Copenhagen, Denmark) was used. The bacteria were cultivated in ordinary nutrient broth. Just prior to the infusion the bacteria were washed in saline and resuspended in 0.9% NaCl, and infused at a concentration of 10^9 live cells/ml. All cats received 1 ml/kg x min for 2 min and then 1 ml/kg x hour for up to three hours.

Experimental procedures

After the operative procedures the cats were allowed to stabilize for 30 min. One minute before the bacterial infusion 0.6 ml bile/kg (obtained from the cat gall-bladder) and 80 mM HCl (volume adjusted to keep the intragastric pressure at 2 cm of water, see above) was instilled into the gastric lumen through the duodenogastric tube. One group of animals (n=8) received yeast CuZn superoxide dismutase (Pharmacia, Uppsala, Sweden); 5 mg as an i.v. bolus 2 min before bacterial infusion, followed by a continuous i.v. infusion of 50 mg/3 hours. In addition, 4 of these cats were also given bovine catalase (Boehringer Ingelheim AB, Skärholmen, Stockholm, Sweden); 5 mg as an i.v. bolus followed by

i.v. infusion 50 mg/3 hours. The drugs were dissolved in 3 ml 0.1 M TRIS HCl buffer with pH 9.5. Another series of animals (n=8) had no treatment and served as controls.

SOD analysis

SOD was determined in terms of its ability to catalyze the disproportionation of $O_2^{\cdot -}$ in alkaline aqueous solution. The disproportionation was directly studied in a spectrophotometer (9). One unit in the assay is defined as the activity that brings about a decay in $O_2^{\cdot -}$ concentration at a rate of $0.1\ s^{-1}$ in 3 ml buffer. It corresponds to 8.3 ng human CuZn SOD, 4.1 ng bovine CuZn SOD and 6 ng yeast CuZn SOD.

Catalase analysis

The rate of H_2O_2 disproportionation was studied at 240 nm in a cuvette containing 10 mM H_2O_2 in 3 ml 10 mM K phosphate, pH 7.4 ± 0.1 mM diethylenetriaminepenta-acetic acid. One unit was defined as the activity that brought about a disproportionation at a rate of $10^{-3}\ s^{-1}$.

Statistical methods

Data are expressed as mean $\pm$ SE if based on five or more observations; otherwise the mean value alone is given. Ordinal scale data are given as median and interquartile range (i.r.). When testing statistical significance the nonparametric methods described by Siegel (10) were used. A p-level <0.05 was considered as significant.

RESULTS

Hemodynamics. Both groups of cats developed a profound hypotension during the bacterial infusion; the end arterial blood pressure at 180 min was 34 ± 22 and 33 ± 21 mmHg in controls (n=8) and SOD/catalase (n=8) treated cats, respectively (fig 1). Only 2 of 8 animals were alive at 180 min in both series and the median survival time was 140 min (range 80 - 180) and 138 min (range 90 - 180) for controls and the treated group.

Cardiac output was 313 ± 31 ml/min and 336 ± 35 ml/min 5 min before bacterial infusion and was reduced to 197 ± 43 and 173 ± 34 ml/min at 150 min in controls (n=3) and treated cats (n=4), re-

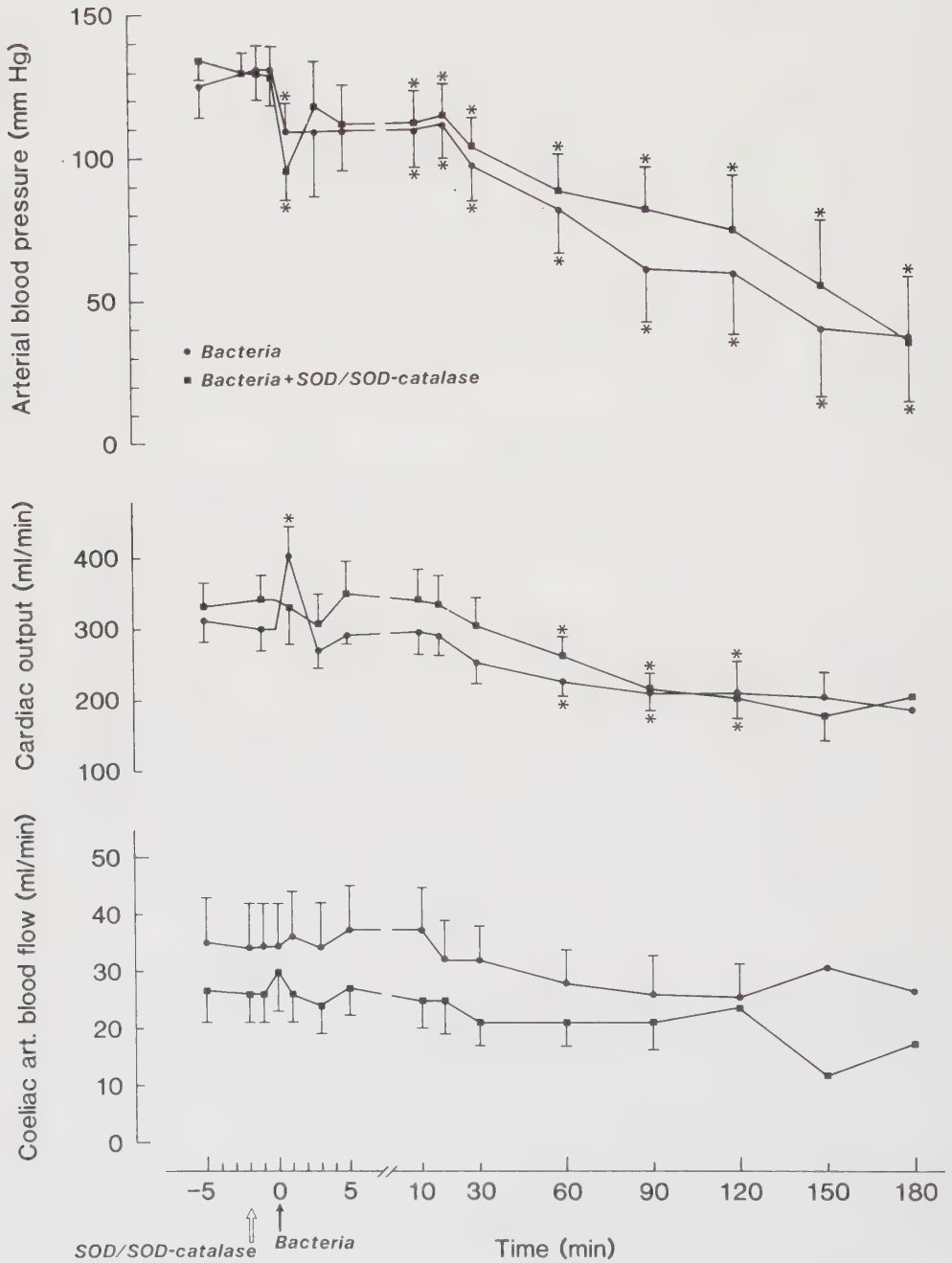


Fig 1: Arterial blood pressure, cardiac output and coeliac artery blood flow in septic cats. * denotes significant difference compared to basal value ($p < 0.05$).

spectively. An increase was seen in controls at one minute with no further significant differences between the groups (fig 1).

Pulmonary arterial blood pressure increased from 14 ± 1 to a maximal value of 34 ± 3 mm Hg within 3 min, normalizing after 15 min in septic cats without treatment. Pulmonary vascular resistance increased to a maximal value of 248 % within 3 min after the start of bacterial infusion and it did not fall to preseptic value during the experiments, being 198 % of the preseptic value at 150 min. These responses were not influenced by SOD or SOD/catalase.

Total gastric blood flow, measured electromagnetically, was 27 ± 6 ml/min in SOD/catalase treated cats and 35 ± 8 ml/min in controls before (-5 min) bacterial infusion. Flow seemed to decrease in both groups, however not statistically significant compared to basal value (fig 1).

The regional flow values, measured by the microsphere technique, are given in table I. Intestinal blood flow data are available in only 6 cats in the control group because of technical failure. There were no changes in gastric mucosal blood flow during the experiments in controls, but a reduced flow was seen at late sepsis in treated cats. In SOD and SOD/catalase treated animals small intestinal mucosal blood flow, small and large total intestinal wall blood flows were reduced at late sepsis. In control series only small intestinal mucosal blood flow decreased at late sepsis. If comparing the two groups, a significant difference was found only in large intestinal flow at late sepsis where controls, thus, had a higher blood flow.

Gastric mucosal damage. All stomachs obtained from control animals were significantly damaged, both macroscopically (bleeding erosions) and at microscopy. The lesion index was 6 or more in all cats (median 8; i.r. 7-8). Four of the SOD/catalase pretreated cats had macroscopically a normal mucosa. Microscopically the lesions in these cats were graded 0 - 1. The median lesion index found in this series was 5 (i.r. 3-7; $p < 0.1$ vs controls). If comparing total number of areas examined (anterior and posterior wall and major curvature of each stomach), however, signi-

TABLE I

Tissue blood flow (ml/min x 100 g)

		Basal	Early sepsis	Late sepsis
Gastric corpus mucosa	S	6.0 $\pm$ 0.8	8.1 $\pm$ 2.0	6.8 $\pm$ 1.5
	S/c	8.3 $\pm$ 2.3	7.7 $\pm$ 2.2	3.6 $\pm$ 0.6*
Gastric antrum mucosa	C	5.4 $\pm$ 0.8	8.0 $\pm$ 3.3	5.8 $\pm$ 2.0
	S/c	12.0 $\pm$ 3.9	13.4 $\pm$ 3.7	4.8 $\pm$ 1.8*
Small intestinal mucosa	C	11.4 $\pm$ 2.6	13.8 $\pm$ 7.6	3.3 $\pm$ 0.8*
	S/c	22.2 $\pm$ 2.8	14.7 $\pm$ 3.9	4.4 $\pm$ 1.3*
Small intestine, total wall	C	14.8 $\pm$ 4.0	28.5 $\pm$ 13.3	19.8 $\pm$ 4.1
	S/c	21.4 $\pm$ 3.1	15.2 $\pm$ 4.3	9.2 $\pm$ 1.9*
Large intestine, total wall	C	21.4 $\pm$ 6.3	21.0 $\pm$ 5.0	18.8 $\pm$ 3.3
	S/c	27.2 $\pm$ 6.3	17.8 $\pm$ 3.9*	7.6 $\pm$ 1.8*

Mean values $\pm$ SE. * denotes statistical significance compared to basal value. C = control (untreated) series; n = 8 for gastric mucosal blood flow and n = 6 for intestinal blood flow values. S/c = SOD and SOD/catalase treated series; n = 8.

ificantly more areas with pronounced lesions (grade 2 - 5) were found in controls compared to SOD or SOD/catalase treated cats ($p < 0.002$) (table II). In both series all lesions seen macroscopically were located in the corpus and fundus area with a normal antrum mucosa.

Small intestinal mucosal damage

The grade of lesion found in each cat is given in table III. Significant damage, i.e. grade 2 - 5 was seen in 50 % of controls and 38 % of treated cats (n.s.). None of the cats that were normotensive at the end of the experiment (two in each group) had any significant lesions of the villi.

TABLE II

Grade lesion in anterior wall, posterior wall and major curvature of the stomach. Grade 0 and 1 means intact superficial epithelium.

■ = control, 0 = SOD or SOD/catalase pretreated cats.

	Grade				
	0	1	2	3	4
Anterior wall		■ 0000	■■■■■ 00	■■ 0	0
Posterior wall	0	■ 000	■■■ 0	■■■ 000	■
Major curvature	0	0000	■■■ 00	■■ 0	■■■

TABLE III

Grade lesion of the small and large intestinal mucosa. Grade 2 - 5 means significant mucosal damage. ■ = control, 0 = SOD or SOD/catalase pretreated animals.

	Grade		
	0 - 1	2 - 3	4 - 5
Small intestine	■■■■ 00000	■■■ 000	■
Large intestine	■■■■■■ 000000	■■ 00	

Large intestinal mucosal damage

Compared to the stomach and small intestine the large intestinal mucosa was more resistant to injury. Only two cats revealed grade 2 lesion or more at microscopy in each group (table II).

Blood tests

SOD in plasma increased from a prebacteriemic value of 20 ± 1 units/ml to 11022 ± 1016 , 15005 ± 2597 and 17215 ± 1672 1, 2 and 2.5 hours from the start of the bacterial infusion in SOD treated cats (n=8). Corresponding values for catalase was (n=4) 0 units/ml (before bacteria), 3830 ± 897 , 5540 ± 1511 and 3263 ± 1209 .

Systemic acidosis was seen in late sepsis. pH decreased from 7.42 ± 0.02 to 7.23 ± 0.04 ($p < 0.05$) in the SOD/catalase treated group and from 7.42 ± 0.03 to 7.25 ± 0.06 ($p < 0.05$) in controls. Polynuclear white blood cells were $6.0 \pm 1.1 \times 10^6/l$, mononuclear $2.0 \pm 0.3 \times 10^6/l$ and platelets $170.0 \pm 30.6 \times 10^6/l$ before septicemia in the treated group and $6.6 \pm 1.0 \times 10^6/l$, $3.6 \pm 0.5 \times 10^6/l$ and $157.2 \pm 32.6 \times 10^6/l$ respectively in controls. White blood cells, mainly polynuclear, declined during bacteremia. Platelets were reduced at early sepsis with a recovery late in the septic period (table IV).

TABLE IV

Polymorphonuclear and mononuclear leucocytes and platelets (% of basal value) in controls (n=8) and SOD/catalase (n=4) or SOD (n=4) pretreated cats before, early and late (see text) after bacterial infusion (mean $\pm$ SE). * denotes statistically significant difference compared to basal value ($p < 0.05$).

	<u>Basal value</u>	<u>Early sepsis</u>	<u>Late sepsis</u>
Polymorphonuclear			
leucocytes			
Control	100	$36 \pm 8^*$	$19 \pm 7^*$
SOD/catalase or SOD	100	$26 \pm 4^*$	$21 \pm 15^*$
Mononuclear			
leucocytes			
Control	100	$46 \pm 14^*$	77 ± 34
SOD/catalase or SOD	100	$42 \pm 8^*$	$45 \pm 14^*$
Platelets			
Control	100	$50 \pm 7^*$	$71 \pm 6^*$
SOD/catalase or SOD	100	$47 \pm 4^*$	$73 \pm 13^*$

DISCUSSION

In the present study live *E.coli* infusion together with topical bile and HCl induced pronounced gastric mucosal lesions. Instillation of bile and HCl without bacteria resulted in no or only minor mucosal damage while the addition of septicemia induced lesions in the gastric mucosa (5). Only 50% of the cats which were infused with CuZn superoxide dismutase alone or together with catalase developed gastric mucosal damage. This indicates that toxic oxygen reduction products contribute to the formation of these lesions in live *E.coli* septicemia. There was less mucosal injury in the small and large bowel and no protection by superoxide dismutase and catalase was observed.

Tissues contain large amounts of superoxide dismutase and catalase (11). In contrast, plasma and other extracellular fluids are much less protected. The catalase activity is negligible (12) and the superoxide dismutase activity is very low (13). It is possible to achieve considerable increases in the superoxide dismutase and catalase activities in the extracellular fluids by injecting the enzymes. The activities achieved in the present investigation were very high and comparable with the intracellular levels (11,14).

Free oxygen radicals have been implicated in the tissue damage seen after experimental ischemia in different organs such as the myocardium, liver, kidney, brain and small intestine (15). One of the intracellular enzymes capable of generating superoxide radicals is xanthine-oxidase which catalyses the oxidation of hypoxanthine to xanthine, the step before formation of uric acid as the end product of ATP catabolism. In these reactions O_2^- is generated. Xanthine-oxidase is inhibited by allopurinol, which has been shown to reduce tissue damage to the small intestinal mucosa in ischemia and reperfusion (3,16). In addition, polymorphonuclear leucocytes are known to be activated in septicemia and septic shock (17). The respiratory burst of phagocytosing leucocytes leads to the release of free radicals into the extracellular space (18). Regardless of the mechanism of generation, oxygen derived free radicals may result in peroxidative injury to membranes of cells, cell organelles like mitochondria (19), and DNA (20).

The exact pathophysiological mechanisms behind gastric stress ulceration is debated. Mucosal ischemia has been proposed as one important factor. This seems to be true in hemorrhagic shock but in septicemia data indicate that mucosal ulcerations develop despite that gastric mucosal blood flow remains in the normal range (5,21,22). Transient ischemia activates xanthine dehydrogenase to its O_2^- producing xanthine oxidase form (23). Whether activation occurs during severe septic shock is not known, but possible. The gastro-intestinal xanthine oxidase appears more easily activated than the enzyme in other organs (23). Polymorphonuclear leucocytes should also be considered as potential sources of toxic oxygen reduction products. However, if the present data are interpreted as an indication of free oxygen radical contribution to acute ulcer formation, one has to conclude that there must be additional pathogenetic mechanisms. Only half of the SOD/catalase or SOD cats had normal mucosa, while the other half had widespread mucosal damage.

To our knowledge there is only one previous report on gastric mucosal ulceration and free radicals (24). In these experiments rats were pretreated with dimethyl sulfoxide, an anti-inflammatory agent which scavenges free radicals. Stress ulceration was induced by cold and restraint. A reduction in macroscopical gastric mucosal lesions was found in the pretreated group.

The small intestinal mucosal lesions seen in E.coli septicemia can be correlated to the systemic arterial hypotension (6) but not to mucosal blood flow (25). Also in intestinal ischemia the mucosal blood flow remains rather normal (26). On the other hand, it has been demonstrated that mucosal damage following ischemia is caused by hypoxia (8). This paradox could be explained by the counter-current exchange mechanism of the small intestinal mucosa as originally proposed by Åhrén and Haglund (8,27). According to this hypothesis the tip of the villus becomes hypoxic in hypotensive states because reduced flow velocity. This will allow a more effective short-circuiting of oxygen in the base of the villus resulting in more pronounced hypoxia at the tip.

In this series of experiments there was no protection of the intestinal mucosa by free oxygen radical scavengers. This indicates

that oxygen derived free radicals do not participate in the pathogenesis of the intestinal mucosal damage in septic shock. This is in agreement with previous reports (3,4) that local intestinal hypotension resulted in significant mucosal damage, which was not affected by SOD or by allopurinol and thus not likely to be caused by the free radicals. However, upon reperfusion, the damage was aggravated and this effect was abolished by SOD or by allopurinol.

The colonic mucosa seems to be less vulnerable to acute mucosal damage in septic shock than the stomach and the small intestine. The low incidence of colonic mucosal damage in the present study prevents us from any conclusions on the pathogenesis of this lesion.

The profound hypotension at late sepsis, the progressive decrease in cardiac output, the initial increase in pulmonary artery blood pressure and the maintained increase of pulmonary vascular resistance were similar to data observed previously in a similar model (28). No effects of SOD or SOD/catalase pretreatment could be found on these variables. The mortality was equal in the two groups. In bled and autotransfused dogs allopurinol increased survival rate (29). Allopurinol in canine endotoxin shock improved hypotension and reduction in cardiac output but survival was unaffected (30). In Nobel-Collip drum trauma shock in rats 2-amino-methyl-4-t-butyl-6-iodosphenol, an oxygen free radical scavenger, doubled survival time (31). The contrary results of these reports and the present can be explained by the different types of shock studied.

To summarize, the present data indicate a potential role of oxygen derived free radicals in the pathogenesis of acute gastric, but not in small intestinal, mucosal damage following i.v. infusion of live *E.coli* in the cat. In untreated control cats this experimental model induced gastric mucosal damage in 100%, small intestinal mucosal damage in 50% and colonic mucosal damage in 20%.

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